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**NEW UNITED STATES UTILITY PATENT APPLICATION**  
 under 37 C.F.R. 1.53(b)

Page 1

Atty. Docket No. 5538/1010

JC794 U.S. PRO  
09/610134  
07/05/00

Assistant Commissioner of Patents  
 Box Patent Applications  
 Washington, D.C. 20231

Express Mail Label No.: EI315834260US

Enclosed herewith is a new patent application and the following papers:

First Named Inventor (or application identifier): Mark Larche & Anthony B. Kay  
 Title of Invention: **METHODS AND COMPOSITIONS FOR DESENSITISATION**

1.  Specification 95 pages (including specification, claims, abstract) / 32 claims ( 17 independent)  
 Insert on Page 1 of the specification –“This is a continuation of PCT/GB99/00080, filed January 11, 1999” \_\_\_\_\_.
2.  Declaration/Power of Attorney is:
  - attached in the regular manner (unexecuted).
  - NOT included, but deferred under 37 C.F.R. § 1.53(f).
3.  16 Distinct sheets of  Formal  Informal Drawings
4.  Preliminary Amendment.
5.  Information Disclosure Statement
  - Form 1449
  - A copy of each cited prior art reference
6.  Assignment with Cover Sheet.
7.  Priority is hereby claimed under 35 U.S.C. § 119 based upon the following application(s):

Country	Application Number	Date of Filing (day, month, year)
GB	9800445.0	9/1/98
GB	9820474.6	21/9/98

Priority is hereby claimed under 35 U.S.C. § 120 based upon the following application(s):

Country	Application Number	Date of Filing (day, month, year)
US	PCT/GB99/00080	11/1/99

8.  Priority document(s).
9.  Statement Claiming Small Entity Status.

**NEW UNITED STATES UTILITY PATENT APPLICATION**  
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Atty. Docket No. 5538/1010

10.  Microfiche Computer Program (Appendix).

11.  Nucleotide and/or Amino Acid Sequence Submission.  
 Computer Readable Copy.  
 Paper Copy (identical to computer copy).  
 Statement verifying identity of above copies.

12. Calculation of Fees:

FEES FOR	EXCESS CLAIMS	FEE	AMOUNT DUE
Basic Filing Fee (37 C.F.R. § 1.16(a))			\$790.00
Total Claims in Excess of 20 (37 C.F.R. § 1.16(c))	12	22.00	\$264.00
Independent Claims in Excess of 3 (37 C.F.R. § 1.16(b))	14	82.00	\$1,148.00
Multiple Dependent Claims (37 C.F.R. § 1.16(d))	Y	270.00	\$270.00
Subtotal - Filing Fee Due			\$2,472.00
	REDUCE BY (%)   (\$)		
Reduction by 50%, if Small Entity (37 C.F.R. §§ 1.9, 1.27, 1.28)	0.50		\$1,236.00
<b>TOTAL FILING FEE DUE</b>			\$0.00
Assignment Recordation Fee (if applicable) (37 C.F.R. § 1.21(h))	0	40.00	\$0.00
<b>GRAND TOTAL DUE</b>			<b>\$1,236.00</b>

13. PAYMENT is:

included in the amount of the GRAND TOTAL by our enclosed check. A general authorization under 37 C.F.R. § 1.25(b), second sentence, is hereby given to credit or debit our Deposit Account No. 19-0733 for the instant filing and for any other fees during the pendency of this application under 37 C.F.R. §§ 1.16, 1.17 and 1.18.

not included; will be paid with response to Notice of Missing Parts.

14. All correspondence for the attached application should be directed to:

PALMER AND DODGE, LLP  
One Beacon Street, Boston, MA 02108  
Telephone: (617) 573-0100  
Facsimile: (617) 227-4420

15. Other: \_\_\_\_\_

Date: July 3, 2000

By:   
Kathleen M. Williams  
Reg. No. 34,380

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee:

Mark Larche and Anthony B. Kay

Serial No.:

Not Assigned Yet

{A Continuation of PCT/GB99/00080 filed January 11, 1999}

Filed:

Concurrent Herewith

Entitled:

METHODS AND COMPOSITIONS FOR DESENSITISATION

Attorney Docket No.:

5538/1010

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS**

**(37 CFR 1.9(d) AND 1.27(d)) -SMALL BUSINESS CONCERN**

I hereby declare that I am an official empowered to act on behalf of the small business concern identified below:

Name of Organization: CIRCASSIA LIMITED

Address of Organization: 90 Fetter Lane, London EC4A 1JP

UNITED KINGDOM

I hereby declare that the small business concern identified above qualifies as a small business concern as defined in 37 C.F.R. § 1.9(d) for purposes of paying reduced fees under § 41(a) and (b) of Title 35, United States Code, with regard to the invention entitled

by inventor(s) Mark Larche and Anthony B. Kay

described in

[X] the specification filed herewith

[ ] Application Serial No. \_\_\_\_\_, filed \_\_\_\_\_.

[ ] Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern with regard to the above-identified invention.

If the rights held by the above identified small business concern are not exclusive, each individual concern or organization having rights in the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR § 1.9(d), or a nonprofit organization under 37 CFR § 1.9(e).

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TITLE IN ORGANIZATION

90 Fetter Lane, London E4A 1JP , United Kingdom

ADDRESS

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SIGNATURE

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DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Mark Larche and Anthony B. Kay

Serial No.: Not Yet Assigned

Filed: Concurrent Herewith  
[A Continuation of PCT/GB99/00080 filed Januay 11, 1999]

Entitled: METHODS AND COMPOSITIONS FOR DESENSITISATION

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Assistant Commissioner for Patents & Trademarks  
Washington, D.C. 20231

Preliminary Amendment

Sir:

Kindly preliminarily amend the specification and claims as follows:

In the specification: Insert on page 1 of the specification: "**This is a continuation of PCT/GB99/00080, filed January 11, 1999.** \_\_\_\_\_ .

In the claims:

Cancel claims 13 and 14.

Amend claim 4 by deleting "any one of Claims 1 to 3" and substituting therefore – claim 1--.

Amend claim 5 by deleting "any one of Claims 1 to 4" and substituting therefore – claim 1--.

Amend claim 15 by deleting "any one of claims 1 to 5" and substituting therefore –claim 1—and by deleting "or a use according to claim 13 or 14".

Amend claim 16 by deleting "or a use according to claim 13 or 14".

Amend claim 19 by deleting "or 19".

Amend claim 20 by deleting "any one of claims 17 to 19" and substituting therefore – claim 17-.

Amend claim 21 by deleting "any one of claims 17 to 20" and substituting therefore – claim 17-.

Amend claim 22 by deleting "any one of claims 17 to 21" and substituting therefore – claim 17-.

Amend claim 23 by deleting "any one of claims 17 to 22" and substituting therefore – claim 17-.

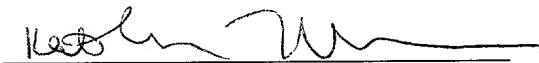
Amend claim 24 by deleting "any one of claims 17 to 23" and substituting therefore – claim 17-.

Amend claim 30 by deleting "or 29".

Amend claim 31 by deleting "any one of claims 28 to 30" and substituting therefore – claim 28-.

Respectfully submitted,

7/5, 2000  
Dated

  
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## METHODS AND COMPOSITIONS FOR DESENSITISATION

The present invention relates to methods and compositions for desensitising patients who are hypersensitive to particular allergens, 5 especially polypeptide allergens. Moreover, the invention relates to immunological vaccines which may be used to prevent and/or treat conditions involving hypersensitivity to allergens.

The ability of the immune system to elicit a response to a particular 10 molecule depends critically upon its ability to recognise the presence of an antigen. Classically, the term antigen has been associated with the ability of a molecule to be an antibody generator *via* induction of B-cells. It is now known, however, that T cells also possess the ability to recognise antigens. T-cell antigen recognition requires antigen presenting cells 15 (APCs) to present antigen fragments (peptides) on their cell surface in association with molecules of the major histocompatibility complex (MHC). T cells use their antigen specific T-cell receptors (TCRs) to recognise the antigen fragments presented by the APC. Such recognition acts as a trigger to the immune system to generate a range of responses to 20 eradicate the antigen which has been recognised.

T lymphocytes have been implicated in the pathogenesis of a wide variety 25 of diseases involving immune recognition of antigens derived both from the internal (host) and external environments. Autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythematosus arise from the recognition by the immune system of host, or self, antigens.

Recognition of external antigens by the immune system of an organism, 30 such as man, can in some cases result in diseases, known as atopic conditions. An example of the latter are the allergic diseases including

asthma, atopic dermatitis and allergic rhinitis. In this group of diseases, B lymphocytes generate antibodies of the IgE class (in humans) which bind externally derived antigens, which are referred to in this context as allergens, since these molecules elicit an allergic response. Production of 5 allergen-specific IgE is dependent upon T lymphocytes which are also activated by (are specific for) the allergen. Allergen-specific IgE antibodies bind to the surface of cells such as basophils and mast cells by virtue of the expression by these cells of surface receptors for IgE. Crosslinking of surface bound IgE molecules by allergen results in 10 degranulation of these effector cells causing release of inflammatory mediators such as histamine, 5-hydroxytryptamine and lipid mediators such as the sulphidoleukotrienes. In addition to IgE-dependent events, certain 15 allergic diseases such as asthma are characterised by IgE-independent events. It has been demonstrated that the induction of the late phase reaction is an IgE-independent event which is dependent upon the activation of allergen-specific T lymphocytes.

Allergic IgE-mediated diseases are currently treated with agents which provide symptomatic relief or prevention. Examples of such agents are 20 anti-histamines,  $\beta_2$  agonists, and glucocorticosteroids. In addition, some IgE-mediated diseases are treated by desensitisation procedures that involve the periodic injection of allergen components or extracts. Desensitisation treatments may induce an IgG response that competes with 25 IgE for allergen, or they may induce specific suppressor T cells that block the synthesis of IgE directed against allergen. This form of treatment is not always effective and poses the risk of provoking serious side effects, particularly general anaphylactic shock. This can be fatal unless recognised immediately and treated with adrenaline. A therapeutic treatment that would decrease or eliminate the unwanted allergic-immune 30 response to a particular allergen, without altering the immune reactivity to

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other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

Asthma can be provoked by inhalation of allergen in the clinical  
5 laboratory under controlled conditions. The response is characterised by an early asthmatic reaction (EAR) followed by a delayed-in-time late asthmatic reaction (LAR) (See *Allergy and Allergic Diseases* (1997), A.B. Kay (Ed.), Blackwell Science, pp 1113 to 1130). The EAR occurs within minutes of exposure to allergen, is maximal between 10 and 15 min and  
10 usually returns to near baseline by 1 hour. It is generally accepted that the EAR is dependent on the IgE-mediated release of mast cell-derived mediators such as histamine and leukotrienes. In contrast the LAR reaches a maximum at 6-9 hours and is believed to represent, at least in part, the inflammatory component of the asthmatic response and in this sense has  
15 served as a useful model of chronic asthma.

The late asthmatic response is typical of responses to allergic stimuli collectively known as late phase responses (LPR). LPR is seen particularly in the skin and the nose following intracutaneous or intranasal  
20 administration of allergens.

Using cat allergic individuals (rhinitic and asthmatic), Norman *et al* (1996) *Am. J. Respir. Crit. Care Med.* 154:1623-8 attempted to induce the counterpart of murine experimental T cell tolerance by subcutaneous  
25 injection of "T cell reactive peptides" (termed IPC1 and IPC2) in humans. Peptides were designed on the basis of patterns of epitope recognition of short overlapping peptides by *Fel d 1* reactive T cell lines. It was found that peptides derived from chain 1 gave greater proliferative responses than chain 2, with the majority of activity being associated in the N terminal region of chain 1. IPC1 and IPC2 were considerably longer (27  
30

amino acids each) than previously defined T-cell epitopes. This may have been partly responsible for immediate (presumed IgE-mediated) reactions in some patients following administration (Norman *et al*, Op. Cit.). Large peptide doses (4 x 750 µg) were required to achieve minimal clinical 5 efficacy. The choice of peptides for therapy was based upon reactivity of secondary T-cell lines derived from a large number of cat-allergic individuals and did not take into account primary T-cell reactivity (ie *ex vivo*), which may be more sensitive, or MHC class II haplotype.

10 Norman *et al* reported a number of adverse hypersensitivity reactions including respiratory, and other allergic, symptoms. As stated, some had a rapid time of onset ie with 10 minutes whereas others were not observed until several hours after IPC1/IPC2 administration (although there was no local redness or swelling at the site of injection). These results have been 15 interpreted as indicating the unsuitability of the peptides for immunotherapy, the production of a LPR being considered to be undesirable (Wheeler & Drachenberg (1997) *Allergy* 52:602-612).

20 WO 92/11859 describes a method of reducing the immune response to an allergen in which a non-allergen derived, non-stimulating peptide which binds to specific MHC class II molecules of APCs is used to inhibit T-cell response to particular allergens.

25 WO 91/06571 purports to disclose peptides derived from human T-cell reactive feline protein which can be used in the diagnosis, treatment or prevention of cat allergy.

30 WO 94/24281 relates to peptides and modified peptides of the major house dust mite allergens. The modified peptides have the intent of reducing the level of undesirable side effects associated with desensitising therapies.

We have observed that peptide allergens used in immunotherapy associate with particular MHC types in patients. Moreover, successful desensitisation of patients is achieved where a peptide allergen is used  
5 which is capable of giving an initial LPR in an individual to whom it is administered.

The MHC complex is a genetic locus made up of a number of genes which encode MHC molecules. MHC molecules are also known as Human  
10 Leucocyte Antigens (HLA).

Each individual inherits a number of MHC genes from each parent and the genes are referred to collectively as the individual's haplotype. This is a genetic term referring to the genes rather than the molecules they encode.  
15 Although the term "haplotype" should, strictly speaking, be used to describe the genes inherited from one parent, it is generally used to include genes from both sets of parents. Where the term is used in this patent specification it is given this general meaning unless the context suggests the stricter meaning.

20 A first aspect of the invention provides a method of desensitising a patient to a polypeptide allergen the method comprising administering to the patient a peptide derived from the allergen wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the  
25 peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

Restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide by, for example, T cell reactivity to the  
30 peptide. By "MHC Class II molecule possessed by the patient" is meant

the particular type which type, of course, may be possessed by other individuals which have the genes that encode the particular type of MHC Class II molecule.

5 By a "peptide derived from the allergen" we include the meaning that the peptide is chemically derived from the polypeptide allergen, for example by proteolytic cleavage and we also include the meaning that the peptide is derived in an intellectual sense from the polypeptide allergen, for example by making use of the amino acid sequence of the polypeptide allergen and  
10 synthesising peptides based on the sequence. Peptides may be synthesised using methods well known in the art, some of which are described in more detail below.

By "peptide" we include not only molecules in which amino acid residues  
15 are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Mézière *et al* (1997) *J. Immunol.* 159, 3230-3237, incorporated herein by reference. This approach involves making pseudopeptides containing  
20 changes involving the backbone, and not the orientation of side chains. Mézière *et al* (1997) show that, at least for MHC class II and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

25

Similarly, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the C $\alpha$  atoms of the amino acid residues is used; it is particularly preferred if the linker moiety has substantially the same charge distribution and  
30 substantially the same planarity as a peptide bond.

It will be appreciated that the peptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

5

By "restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide" we mean that the peptide is able to bind to a particular MHC Class II possessed by the patient. That is not to say that a particular peptide cannot bind to another MHC Class II molecule.

10 Peptides are generally only recognised in the context of a "self" MHC molecule, thus recognition of MHC-bound peptides by an individual's T cells is generally restricted by the MHC molecules expressed by the individual molecule.

15 Although binding to the given MHC Class II molecule may be demonstrated directly using suitable samples from the patient, whether or not a particular peptide can bind to a particular MHC Class II molecule (ie is restricted by a particular Class II molecule) can readily be determined *in vitro* using methods well known in the art, some of which are disclosed  
20 below.

Determination of the MHC Class II haplotype of the patient or the identification of particular MHC Class II genes possessed by the patient can readily be determined using any suitable method as is well known in  
25 the art, including the PCR-based methods described more fully below for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Determination of the MHC Class II haplotype indicates which MHC molecules are expressible by an individual.

30 By "late phase response" we include the meaning as set forth in *Allergy*

and Allergic Diseases (1997) A. B. Kay (Ed.), Blackwell Science, pp 1113- 1130. The late phase response may be any late phase response (LPR). Preferably, the peptide is able to induce a late asthmatic response (LAR) or a late rhinitic response, or a late phase skin response or a late phase ocular response. Whether or not a particular peptide can give rise to a LPR can be determined using methods well known in the art; a particularly preferred method is that described in Cromwell O, Durham SR, Shaw RJ, Mackay J and Kay AB. Provocation tests and measurements of mediators from mast cells and basophils in asthma and allergic rhinitis. In: Handbook of Experimental Immunology (4) Chapter 127, Editor: Weir DM, Blackwell Scientific Publications, 1986. Not all individuals who possess the particular MHC Class II molecule would experience a LPR following the administration of allergen or allergen-derived peptides since generation of the LPR is dependent upon prior sensitisation to the allergen in question.

Thus, preferably, the peptide is able to induce a LPR in an individual who possesses the said MHC Class II molecule and who has been sensitised to the allergen in question. Whether or not an individual has been sensitised to the allergen in question may be determined by well known procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radio-allergosorbent test (RAST) for measurement of allergen specific IgE.

Preferably, the peptide is included in a composition containing a plurality of peptides derived from the said allergen. The peptides in the composition may or may not be multiple overlapping peptides (MOPs) derived from the polypeptide allergen. The plurality of peptides may be derived from the whole of the polypeptide allergen and therefore the peptides span the whole of the polypeptide chain or chains of the allergen.

However, they may be derived from only portions of the polypeptide allergen such that some portions of the polypeptide allergen are not represented in the plurality of peptides (for example, as is shown below, some peptides derived from an allergen may not be very soluble in 5 aqueous solution and so may not be useful and other peptides may not show restriction to MHC Class II molecules). MOPs or any peptides derived from the allergen and present in the composition can be designed by reference to the amino acid sequence of the polypeptide allergen. Typically, the peptides are at least seven amino acid residues. Typically, 10 the peptides would be between around 14 to 18 amino acid residues in length. It is preferred that the peptides have a reduced ability to bind IgE compared to longer peptides containing the same sequence. It is particularly preferred if the peptides are substantially incapable of binding IgE. Typically, when the MOPs overlap, the overlap is around one amino 15 acid residue. This is particularly useful when the MOPs are used in *in vitro* T cell assays in order to identify MHC-binding peptides which may then be screened for their ability to induce LPR in an individual. More details of screening procedures are given below.

20 MHC Class II molecules are encoded by MHC Class II genes. There are at least three loci (DR, DQ and DP) that encode MHC Class II molecules, and each individual has two copies of each locus. These loci exhibit considerable genetic diversity and the preponderance of different MHC Class II genes (alleles) varies. The approximate frequencies of various 25 MHC Class II genes (alleles) from a normal (disease free) population of people in England is described in Haworth S, Sinnott P, Davidson J & Dyer P. Caucasian England Normal In: HLA Typing 1997, Eds: Terasaki, PI and Gjertson, DW, Publishers: UCLA tissue typing laboratory, incorporated herein by reference.

For DR molecules, the most common in the Caucasian population are those that can be classified DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR51, DR52 and DR53.

5 For DP molecules, the most common are DPB1\*0201, DPB1\*0301 and DPB1\*0401.

For DQ molecules, the most common are DQB1\*0201, DQB1\*0301, DQB1\*0501, DQB1\*0601 and DQB1\*0602.

10

It is particularly preferred if the plurality of polypeptides administered to the patient includes peptides for which restriction to MHC Class II molecules can be demonstrated. It is particularly preferred if the plurality of peptides administered to the patient includes peptides for which 15 restriction to the MHC Class II DR molecules DR2, DR3, DR4, and DR7 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC Class II DR molecules DR1, DR5 and DR6 can be demonstrated.

20

It is also particularly preferred if the plurality of peptides administered to the patient includes peptides for which restriction to the MHC Class II DR molecules DR51, DR52 and DR53 has been demonstrated.

25 It is also particularly preferred if the plurality of peptides administered to the patient includes peptides for which restriction to the MHC Class II DP molecules DPB1\*0201, DPB1\*0301 and DPB1\*0401 can be demonstrated.

30 It is also particularly preferred if the plurality of peptides administered to

the patient includes peptides for which restriction to the MHC Class II DQ molecules DQB1\*0301 and DQB1\*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC 5 Class II DQ molecules DQB1\*0201, DQB1\*0501 and DQB1\*0602 can be demonstrated.

It is preferred if the plurality of peptides includes only a single peptide for which restriction to a particular MHC Class II molecule can be 10 demonstrated.

Restriction to a particular Class II molecule can be demonstrated as has been described above and is described in more detail below. It will be appreciated that it may not be possible to derive a peptide for which 15 restriction to a particular Class II molecule can be demonstrated; for example, a particular polypeptide allergen may not contain a T cell epitope which can be presented by every MHC Class II molecule. In this case, of course, such a peptide is not present in the plurality of peptides derived from the polypeptide allergen.

20 By "desensitising a patient to a polypeptide allergen" is meant inhibition or dampening of allergic tissue reactions induced by allergens in appropriately sensitised individuals. It will be appreciated that whether or not a patient is sensitive to a particular polypeptide allergen can be 25 assessed using well known procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radio-allergosorbent test (RAST) for measurement of allergen specific IgE, and whether or not a particular patient is one who is expected to benefit from treatment may be 30 determined by the physician based, for example, on such tests.

Administration of the peptide (such as the composition containing a plurality of peptides) may be by any suitable method, some of which are described below in more detail. Suitable amounts of the peptide may be determined empirically, but typically are in the range given below. As is 5 described in a further aspect of the invention below, the invention also includes a method of determining an initial dose of peptide which is suitable to administer to the patient. A single administration of the peptide may be sufficient to have a beneficial effect for the patient, but it will be appreciated that it may be beneficial if the peptide is administered more 10 than once, in which case typical administration regimes may be, for example, once or twice a week for 2-4 weeks every 6 months, or once a day for a week every four to six months.

A second aspect of the invention provides a composition comprising a 15 plurality of peptides derived from a polypeptide allergen wherein for at least one of the peptides in the composition restriction to a MHC Class II molecule can be demonstrated and the composition is able to induce a late phase response in an individual possessing the given MHC Class II molecule. Preferably, at least one peptide is present in the composition 20 for which restriction to each of MHC Class II DR molecules DR2, DR3, DR4 and DR7 can be demonstrated, provided of course that such peptides can be derived from the allergen.

Also preferably the composition may include peptides for which restriction 25 to any one or more of the MHC Class II DR molecules DR1, DR5 and DR6 can be demonstrated.

Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class II DR molecules DR51, DR52 and 30 DR53 has been demonstrated.

Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class II DP molecules DPB1\*0201, DPB1\*0301, and DPB1\*0401 can be demonstrated.

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Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class DQ molecules DQB1\*0301 and DQB1\*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to 10 any one or more of the MHC Class II DQ molecules DQB1\*0201, DQB1\*0501 and DQB1\*0602 can be demonstrated.

These preferences are all with the proviso that for any particular allergen it may not be possible to derive a peptide for which restriction to a 15 particular Class II molecule can be demonstrated.

Although the composition (or a peptide within the composition) is able to induce a LPR in an individual possessing the given MHC Class II molecule (and as described below in more detail suitable compositions and 20 peptides may be identified by their ability to induce a LPR), it should be appreciated that when the composition (or a peptide within the composition) is used to treat a patient it is preferable that a sufficiently low concentration of the composition or peptide is used such that no observable LPR will occur but the response will be sufficient to partially 25 desensitise the T cells such that the next (preferably higher) dose may be given, and so on. In this way the dose is built up to give full desensitisation but often without ever inducing a LPR in the patient (although, of course, the composition or peptide is able to do so at a higher concentration than is administered. It will be appreciated further, 30 and as discussed in more detail below, induction of LPR in an individual

is particularly useful in selecting appropriate compositions and peptides but is not essential in the clinical efficacy and treatment stages.

It will be appreciated that the composition may contain as many or as few  
5 peptides derived from the polypeptide allergen as will make it useful.  
Although in one embodiment of the method of desensitising the patient of  
the first aspect of the invention a single peptide may be administered to the  
patient wherein the peptide demonstrates restriction to a MHC Class II  
molecule possessed by the patient and the peptide is able to induce a late  
10 phase response in an individual who possesses the said MHC Class II  
molecule, it is preferred if the composition of the second aspect of the  
invention contains sufficient number of peptides, each of which  
demonstrate restriction to a particular MHC Class II molecule and which  
are able to induce a late phase response in an individual who possesses the  
15 said MHC Class II molecule, such that for at least 75% of the population a  
peptide is present in the composition which is MHC Class II restricted and  
which is capable of inducing a late phase response in an individual with an  
appropriate restricted MHC Class II molecule. More preferably the  
composition contains sufficient peptides such that for at least 80% of the  
20 population (and still more preferably at least 85%, or yet still more  
preferably 90% of the population) a peptide is present in the composition  
which is MHC Class II restricted and which is capable of inducing a late  
phase response in an individual with an appropriate restricted MHC Class  
II molecule.

25

In a particularly preferred embodiment, the composition contains (as the  
only polypeptide allergen-derived peptide components of the composition)  
peptides which are MHC Class II restricted and which are capable of  
inducing a LPR in an individual who possesses the given MHC Class II  
30 molecule. Preferably, the composition contains as the only polypeptide

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allergen-derived peptide components a sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a LPR in an individual who possesses the said MHC Class II molecule, such that for at least 75% of the population a 5 peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a LPR in an individual with an appropriate restricted MHC Class II molecule.

It is well known that the frequency of particular MHC Class II molecules 10 in a population varies with ethnic groups, and that for at least some ethnic groups the frequency of particular MHC Class II molecules is known (see, for example, HLA Typing 1997, *supra*). For example, the frequency of particular MHC Class II molecules is different in the Caucasian population compared to the Mongoloid population or Negroid population and so on. 15 It will readily be appreciated that the polypeptide allergen-derived peptides to be included in a composition of the invention may be selected according to the ethnic group to which the patient belongs. For example, compositions of the invention may readily be prepared for desensitisation to a particular polypeptide allergen by reference to the MHC Class II gene 20 frequencies in the Caucasian or Mongoloid or Negroid populations.

A third aspect of the invention provides a composition of the second aspect of the invention packaged and presented for use in medicine. In particular, the composition will be packaged and presented with an 25 indication of who may be treated (in particular who may benefit from being treated) with the composition including, if desirable, an indication of the MHC Class II molecules to which the peptides within the composition are restricted.

30 It will be appreciated that the composition of the second aspect of the

invention is conveniently administered to the patient according to the method of the first aspect of the invention.

A fourth aspect of the invention provides a pharmaceutical formulation  
5 comprising a composition according to the second aspect of the invention and a pharmaceutically acceptable carrier. Suitable ingredients for pharmaceutical formulations are described in more detail below.

A fifth aspect of the invention provides the use of a peptide derived from a  
10 polypeptide allergen wherein restriction to a MHC Class II molecule possessed by a patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule in the manufacture of a medicament for desensitising a patient to said polypeptide allergen.

15

A sixth aspect of the invention provides the use of a composition according to the second aspect of the invention in the manufacture of a medicament for desensitising a patient to said polypeptide allergen.

20 It will be appreciated that with respect to the method of the first aspect of the invention it may be desirable to determine which MHC Class II molecules the patient possesses in order to select an appropriate peptide or composition to administer to the patient. (It will be appreciated that this may be determined by determining the MHC haplotype of the individual  
25 by genetic means.) This is particularly desirable when the administration of a single peptide is contemplated. However, it will also be appreciated that when a composition is used which contains sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a late phase response in an  
30 individual who possesses the said MHC Class II molecule, such that for at

least 75% (or more preferably 80%, or 85% or 90%) of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with an appropriate restricted MHC Class II molecule, then it may not be 5 necessary or desirable to type the patient to determine which MHC Class II molecules he or she possesses.

The polypeptide allergen may be any polypeptide allergen, some of which are described in more detail below.

10

A seventh aspect of the invention provides a method of selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to a polypeptide allergen capable of eliciting an allergic response in the patient, which patient possesses a particular MHC Class II molecule, the method 15 comprising the steps of (1) selecting a candidate peptide derived from the polypeptide allergen, (2) determining whether the candidate peptide demonstrates restriction to the said MHC Class II molecule, and (3) determining whether the candidate peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

20

The candidate peptide may be any peptide derived from the polypeptide allergen and is, conveniently, a polypeptide in the size range described elsewhere as being a suitable size of a peptide for use in immunotherapy.

25 Whether or not the candidate demonstrates restriction to the said MHC Class II molecule may be determined by any suitable method such as those well known in the art, some of which are described in the Examples.

30 Whether or not the candidate peptide is able to induce a LPR can be determined by the methods described herein and which are well known in

the art. It is particularly preferred if step (2) is carried out prior to step (3) and only candidate peptides which demonstrate restriction to the particular MHC Class II molecules are selected for testing in step (3).

5    It is particularly preferred that the individual in step (3) is an appropriately sensitised individual; that is to say an individual who has been sensitised previously to the allergen in question. It is those peptides which are capable of inducing a LPR and which demonstrate restriction to the particular MHC Class II molecule which are selected as an immuno-  
10   therapeutic agent.

Determination of whether the candidate peptide demonstrates restriction to the said MHC Class II molecules may conveniently be done using a suitable T cell activation assay.

15   Thus, in one preferred embodiment the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen capable of eliciting an allergic response in the patient which patient possesses a particular MHC Class II haplotype,  
20   comprising the steps of:

a)   administering a candidate peptide to an individual who possesses the same said MHC Class II molecule as the patient and determining whether the peptide induces a late phase response; and  
25   b)   selecting a peptide capable of inducing a late-phase response as an immunotherapeutic agent.

The individual to whom the candidate peptide is administered for the  
30   purpose of determining whether the peptide induces a LPR may or may

not be the patient.

In an eighth aspect, the invention provides a method for testing for candidate peptides for further selection according to the preferred embodiment discussed immediately above of the invention, comprising the steps of:

- a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation of an individual's T-cells;
- 10 b) tissue-typing the individual to determine MHC type;
- c) determining the MHC molecule(s) bound by each candidate peptide; and
- 15 d) selecting a peptide or peptides satisfying part (a) above and capable of binding to an MHC type possessed by the individual, for use as a candidate peptide in a method according to the preferred embodiment discussed immediately above.

In a ninth aspect, the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen comprising the steps of:

- 25 a) tissue-typing the patient to determine MHC Class II type; and
- b) selecting, from a database of peptides which are known to bind to particular MHC molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides  
30 capable of binding to the MHC Class II molecules possessed by the

individual.

Preferably, the individual is an appropriately sensitised individual who has been sensitised previously to the allergen in question.

5

In a tenth aspect, the invention provides a database of peptides characterised according to the seventh and eighth aspects of the invention.

TCRs are highly variable in their specificity. Variability is generated, as with antibody molecules, through gene recombination events within the cell. TCRs recognise antigen in the form of short peptides bound to molecules encoded by the genes of the Major Histocompatibility Complex (MHC). These gene products are the same molecules that give rise to "tissue types" used in transplantation and are also referred to as Human Leukocyte Antigen molecules (HLAs) which terms may be used interchangeably within this document. Individual MHC molecules possess peptide binding grooves which, due to their shape and charge are only capable of binding a limited group of peptides. The peptides bound by one MHC molecule may not necessarily be bound by other MHC molecules.

10 As a result of this restricted peptide-MHC binding, T cell receptor recognition of a particular peptide is said to be "restricted" by the MHC molecule to which the peptide is bound. As used herein the term "allergen peptide-binding MHC" will be used to mean the MHC molecule(s) that bind the said allergen or allergen-derived peptide.

15

20

When a protein molecule such as an antigen or allergen is taken up by antigen presenting cells such as B lymphocytes, dendritic cells, monocytes and macrophages, the molecule is enzymatically degraded within the cell. The process of degradation gives rise to peptide fragments of the molecule which, if they are of the appropriate size, charge and shape, may then

30

bind within the peptide binding groove of certain MHC molecules and be subsequently displayed upon the surface of antigen presenting cells. If the peptide/MHC complexes are present upon the antigen presenting cell surface in sufficient numbers they may then activate T cells which bear the  
5 appropriate peptide/MHC-specific T cell receptors.

Due to the polymorphic nature of the MHC, individuals in an outbred population such as man will express different combinations of MHC molecules on their cell surfaces. Since different MHC molecules can bind  
10 different peptides from the same molecule based on the size, charge and shape of the peptide, different individuals will display a different repertoire of peptides bound to their MHC molecules.

Identification of universal MHC-binding peptide epitopes in an outbred  
15 population such as man is more difficult than in inbred animals (such as certain strains of laboratory mice). On the basis of differential MHC expression between individuals and the inherent differences in peptide binding and presentation which this brings, it is unlikely that a single peptide can be identified which will be of use for desensitisation therapy in  
20 man for most diseases unless the association of a particular MHC molecule with that disease is very strong. For example, the HLA-B27 molecule has been shown to have a close relationship with ankylosing spondylitis, where approximately 90% of sufferers express HLA-B27. For some autoimmune diseases, certain disease HLA associations have been  
25 demonstrated eg HLA-DR4 and rheumatoid arthritis, but these associations are much weaker than for ankylosing spondylitis.

In allergic diseases, associations are even weaker if demonstrated at all. For this reason, it is unlikely that therapies centred around a single peptide  
30 (even an immunodominant one) or small numbers of peptides will be

optimally effective as desensitisation therapies. The conclusion drawn in the art where MHC binding allergen epitopes have been identified is that even if an immunodominant epitope is identified, it would appear that it is required to react with a variety of restricted MHCs to be of therapeutic value (see Van Neerven RJJ *et al* (1994) *J Immunol* 152, 4203-4210; Higgins JA *et al* (1994) *J Allerg Clin Immunol* 93, 891-899).

As set forth herein, it has now been observed that a patient may be desensitised to a particular allergen by the administration of a peptide or a composition containing a peptide that is able to bind to at least one MHC molecule of said patient and which is able to induce a LPR in an individual who possesses the same MHC Class II molecule type.

According to the present invention, therefore, the concept of "universal" desensitising peptides is rejected in favour of a selective approach which takes into account tissue type. Nevertheless, it will be appreciated that using a composition containing a plurality of peptides according to the present invention may be "universal" in the sense that a single composition may be used for most of the population, but that this is still selective on the basis that the composition contains peptides which are restricted by a particular MHC Class II molecule.

It can be hypothesised that eosinophil-dependent mucosal tissue damage, including LPR, is under T-cell control. For example, by *in situ* hybridisation the numbers of mRNA positive cells for the Th2-type (IL-4 and IL-5) and eosinophil-active cytokines (IL-3, IL-5 and GM-CSF) were shown to be elevated in asthmatics both at baseline (Robinson *et al* (1992) *N Engl J Med* 326: 298-304) and following allergen-induced LAR (Bentley *et al* (1993) *Am J Respir Cell Mol Biol* 8:35-42). Furthermore IL-4 and IL-5 mRNA co-localised largely to CD4+ T cells (Ying *et al* (1997) *J Immunol* 158:3539-3544). A T cell component of the LAR is also

suggested by the observation that cyclosporin A attenuated the LAR, but not the EAR, provoked by allergen inhalation (Sihra *et al* (1997) *Thorax* 52:447-452). Furthermore a single infusion of anti-CD4 produced significant improvement in lung function in chronic 5 corticosteroid-dependent asthmatics. However it has been difficult to determine whether T cell activation, as an initiating event, leads directly to airway narrowing in asthmatic patients and therefore an asthmatic response.

10 As described herein, it has now been shown that T cells can be selectively activated, and then rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to desensitisation of the patient for a particular allergen. The desensitisation manifests itself as a reduction in response to an allergen or allergen-derived peptide, or preferably an 15 elimination of such a response, on second and further administrations of the allergen or allergen-derived peptide. The second administration may be made after a suitable period of time has elapsed to allow desensitisation to occur; this is preferably any period between one day and several weeks. An interval of around two weeks is preferred.

20

Based on these results, the invention provides a method for desensitising a patient to a polypeptide allergen which comprises the administration to the patient of a peptide specifically selected to induce LPR and subsequent desensitisation in the patient wherein the peptide is restricted by a 25 particular MHC Class II molecule and capable of inducing LPR in an individual who possesses the given MHC Class II molecule to which the peptide is restricted. The peptides for desensitisation may be selected according to whether they induce LPR.—

30 LPR is defined as set forth in *Allergy and Allergic Diseases* (1997) A.B.

Kay (Ed.), Blackwell Science, pp 1113 to 1130, and includes asthmatic, cutaneous and nasal late phase responses as described above.

As noted above, the peptide which is administered may be included in a  
5 composition containing a plurality of peptides derived from the allergen.

Preferably, the peptides are derivatives of the allergen itself, and retain at least one common antigenic determinant of the allergen. "Common antigenic determinant" means that the derivative in question retains at least  
10 one antigenic function of the allergen. Antigenic functions include possession of an epitope or antigenic site that is capable of binding to TCRs which recognise the allergen or fragments thereof. Thus, the peptides provided by the present invention include splice variants encoded by mRNA generated by alternative splicing of a primary transcript  
15 encoding the allergen, amino acid mutants, glycosylation variants and other covalent derivatives of the allergen which retain at least an MHC-binding property of the allergen. Exemplary derivatives include molecules wherein the peptide of the invention is covalently modified by substitution, chemical, enzymatic, or other appropriate means with a  
20 moiety other than a naturally occurring amino acid. Further included are naturally occurring variants of the allergen found in a particular species. Such a variant may be encoded by a related gene of the same gene family, by an allelic variant of a particular gene, or represent an alternative splicing variant of the allergen gene.

25

Derivatives of the allergen also comprise mutants thereof, which may contain amino acid deletions, additions or substitutions, subject to the requirement to maintain at least one feature characteristic of the allergen. Thus, conservative amino acid substitutions may be made to peptides  
30 according to the invention substantially without altering the nature of the

allergen, as may truncations from the N or C termini. Deletions and substitutions may moreover be made to the fragments of the allergen comprised by the invention. Peptides may be produced from a DNA which has been subjected to *in vitro* mutagenesis resulting eg in an addition, 5 exchange and/or deletion of one or more amino acids. Preferably, peptides are produced by peptide synthesis according to known techniques using commercially available peptide synthesisers. Mutations and/or truncations may thus be made by changing the amino acid sequence during the synthesis procedure.

10

Suitable variants capable of binding to TCRs may be derived empirically or selected according to known criteria. Within a single peptide there are certain residues which contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable 15 regions of the T cell receptor (Allen *et al* (1987) *Nature* 327:713-5). Within the residues contributing to T cell receptor interaction, a hierarchy has been demonstrated which pertains to dependency of T cell activation upon substitution of a given peptide residue. Using peptides which have had one or more T cell receptor contact residues substituted with a 20 different amino acid, several groups have demonstrated profound effects upon the process of T cell activation. Evavold & Allen (1991) *Nature* 252:1308-10) demonstrated the dissociation of T cell proliferation and cytokine production. In this *in vitro* model, a T cell clone specific for residues 64-76 of haemoglobin (in the context of I-E<sup>k</sup>), was challenged 25 with a peptide analogue in which a conservative substitution of aspartic acid for glutamic acid had been made. This substitution did not significantly interfere with the capacity of the analogue to bind to I-E<sup>k</sup>. Following *in vitro* challenge of a T cell clone with this analogue, no proliferation was detected although IL-4 secretion was maintained, as was 30 the capacity of the clone to help B cell responses. In a subsequent study

the same group demonstrated the separation of T cell-mediated cytolysis from cytokine production. In this instance, the former remained unaltered while the latter was impaired. The efficacy of altered peptide ligands *in vivo* was initially demonstrated in a murine model of EAE (experimental allergic encephalomyelitis) by McDevitt and colleagues (Smilek *et al* (1991) *Proc Natl Acad Sci USA* 88:9633-9637). In this model EAE is induced by immunisation with the encephalitogenic peptide Ac1-11 of MBP (myelin basic protein). Substitution at position four (lysine) with an alanine residue generated a peptide which bound well to its restricting element ( $A\alpha^uA\beta^u$ ), but which was non-immunogenic in the susceptible PL/JxSJLF1 strain and which, furthermore prevented the onset of EAE when administered either before or after immunisation with the encephalitogenic peptide. Thus, residues can be identified in peptides which affect the ability of the peptides to induce various functions of T-cells.

Advantageously, peptides may be designed to favour T-cell proliferation and induction of desensitisation. Metzler and Wraith have demonstrated improved tolerogenic capacity of peptides in which substitutions increasing peptide-MHC affinity have been made (Metzler & Wraith (1993) *Int Immunol* 5:1159-65). The demonstration that an altered peptide ligand can cause long-term and profound anergy in cloned T cells (Sloan-Lancaster *et al* (1993) *Nature* 363:156-9) is particularly relevant to the applications of such peptide analogues in immunotherapy for diseases such as autoimmunity and allergy, in addition to the induction of host/donor-specific tolerance in transplantation.

Derivatives which retain common antigenic determinants are preferably fragments of the allergen. Fragments of the allergen comprise individual domains thereof, as well as smaller polypeptides derived from the

domains. Preferably, smaller polypeptides derived from the allergen according to the invention define a single epitope of the allergen capable of binding a TCR. Fragments may in theory be almost any size, although smaller fragments are more likely to be restricted to a single MHC molecule and are thus preferred. Preferably, fragments will be between 5 and 50, preferably between 5 and 25, and advantageously about 17 amino acids in length. It is preferred if the peptides do not invoke an IgE response and do not lead to the release of histamine from enriched basophils or mast cell preparations from most sensitised individuals.

10

Candidate peptides potentially capable of inducing LPR in a patient may be preselected in order to maximise the chances of identifying a therapeutically useful peptide in *in vivo* tests. The steps of this aspect of the invention comprise the determination that the peptide is MHC Class II restricted, for example it is capable of causing T-cell proliferation when associated with an MHC molecule present in the patient to be treated. Thus, in a particular embodiment the selection procedure can be broken down into three steps, performed either sequentially (in any order) or together:

20

a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation in an individual's T-cells;

b) tissue-typing the individual to determine MHC Class II type; and

25

c) determining the MHC Class II molecule bound by each candidate peptide.

Steps (a) and (c), in particular, may be combined in a single T-cell activation assay. Preferably, the assay involves the use of cells transfected

to express a particular MHC molecule, and the binding of the peptide to this MHC assessed by its ability to induce T-cell proliferation in the presence of the transfected cells alone. Suitable transfected cells are readily available and can, in any case, be readily made by transfecting the  
5 cloned genes into suitable cell lines.

Preferably, a peptide selected according to the above procedure is tested for its ability to induce LPR in an individual. If LPR is induced, repeated administration will result in desensitisation to the allergen from which the  
10 peptide is derived.

However, once a peptide has been determined to bind a particular MHC Class II type and to be capable of inducing LPR when administered to an individual possessing that MHC Class II type, it can be used to induce  
15 desensitisation to the relevant allergen in substantially any patient possessing the required MHC Class II molecule. Therefore, peptides derived from particular allergens may be characterised according to their binding to particular MHC Class II types and their ability to induce LPR, thus providing a database from which a suitable peptide may be selected  
20 for any given patient upon tissue typing of that patient. Additionally or alternatively, a preparation containing a plurality of MHC-binding peptides capable of inducing LPR may be employed which will be effective in desensitising the majority of sensitised individuals.

25 Thus, in one embodiment antigen presenting cells may be isolated from a patient known to be sensitive to a particular allergen or allergens, and based on the peptide-binding MHC molecules displayed by said cells, a peptide may be selected for use in desensitising said patient by virtue of its ability to bind to at least one MHC molecule. The invention accordingly  
30 provides a method for selecting a peptide for use as an immunotherapeutic

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agent for desensitising a patient to an allergen comprising the steps of:

- a) tissue-typing the patient to determine MHC Class II type; and
- 5 b) selecting, from a database of peptides which are known to bind to particular MHC Class II molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides capable of binding to the MHC Class II molecules possessed by the patient.

10

For the avoidance of doubt, the individual referred to in part (b) above need not necessarily be the same individual as the patient undergoing treatment whom is tissue typed in part (a). In fact, once the MHC Class II restriction of a particular allergen-derived peptide is determined, and it has 15 been determined that the peptide is capable of inducing a LPR in an individual, particularly an appropriately sensitised individual, who possesses the said MHC Class II molecule, there is no requirement to test the ability of the patient's own MHC Class II molecules.

20 Allergens that may be amenable to desensitisation procedures as described herein include the peptides derived or chosen from the list comprising the allergens; Fel d 1 (the feline skin and salivary gland allergen of the domestic cat *Felis domesticus* - the amino acid sequence of which is disclosed in WO 91/06571), Der p I, Der p II, Der fI or Der fII (the 25 major protein allergens from the house dust mite *dermatophagoides* - amino acid sequences disclosed in WO 94/24281).

The invention is applicable substantially to any allergen, including allergens present in any of the following: grass, tree and weed (including 30 ragweed) pollens; fungi and moulds; foods eg fish, shellfish, crab lobster,

peanuts, nuts, wheat gluten, eggs and milk; stinging insects eg bee, wasp and hornet and the chironomidae (non-biting midges); spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cows, pigs,  
5 sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; and protein detergent additives.

Where the allergen is an insect protein, the peptides may be selected from the group comprising: housefly, fruit fly, sheep blow fly, screw worm fly,  
10 grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of *Tenibrio molitor* beetle. All these being insect allergens, they are of particular relevance to allergic problems arising in the workplace.

15 Where the allergen is the Fel d 1 allergen, useful peptides may preferably comprise a sequence as shown in any one of SEQ ID Nos. 1 to 3. Particular preferred peptides for use in the methods of the invention are those with the sequence given in SEQ ID Nos. 1 or 2 or 3. Preferred compositions of the invention include those that contain the peptides with  
20 the sequence given in SEQ ID Nos. 1, 2 and 3, and compositions containing the MHC Class II-restricted peptides of the thirteen peptides described in Example 7 and for which can be determined a LPR in an individual possessing appropriate MHC Class II molecules.

25 A database according to the invention includes information on the MHC Class II molecule(s) bound by peptides in the database and the ability of the peptides to induce a LPR in patients possessing such MHC Class II molecule(s). Thus, the database allows a practitioner to select peptides capable or potentially capable of eliciting a LPR and therefore  
30 desensitisation in a particular patient on the basis of that patient's tissue

type.

The invention moreover provides a peptide listed in a database according to the invention, for use in therapy. Preferably, such peptides are useful  
5 in methods for desensitising patients to allergens in accordance with the methods set forth herein. Peptides to be included in the database, and  
- peptides which may be useful either individually or as a mixture in a composition of the invention may readily be selected by the methods of the invention from polypeptide allergens whose polypeptide sequences, or  
10 reference to polypeptide sequences, are given in Example 6.

The MHC molecules expressed on APCs which bind peptides derived from a specific allergen may be identified by methods known in the art, such as T cell proliferation studies with MHC blocking antibodies, and  
15 PCR techniques, for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Thus, antigen-presenting cells, expressing a variety of MHC molecules may be incubated with allergen and T cells and the latter observed for proliferation. Addition of antibodies to specific MHC classes may then be made in repeat  
20 incubations in order to identify the restricted MHC in respect of the allergen being tested. See Van Neerven RJ et al (1994) *Immunol* 82:351-356, and Yssel H et al (1992) *J Immunol* 148:738-745.

Alternatively, cells presenting a single MHC Class II type, for example  
25 cells such as fibroblast cells transfected with the genes encoding an MHC Class II molecule, may be incubated with individual peptides for which T-cell clones or lines are known to be specific. Culturing of such T-cell clones or lines with peptide presented by the appropriate MHC Class II molecule will lead to T-cell proliferation. T cell proliferation is not the  
30 only indicator that a particular peptide binds to a particular MHC Class II

molecule on an APC. Other indicators include the secretion of measurable soluble products such as cytokines, changes in intracellular calcium levels, and other means of measuring T cell activation which are well known in the art.

5

Preferred fibroblasts for use in this aspect of the invention include human or murine fibroblasts, particularly L-cells.

10 The latter method may be used in a combinatorial approach, in which groups of peptides may be tested together and effective peptides identified by standard combinatorial techniques.

15 Specific epitopes of the allergen or peptide derived therefrom that bind to at least one MHC Class II molecule may then be identified by standard procedures and used in desensitisation procedures as described herein. Accordingly, the invention provides peptides when selected according to the foregoing aspects of the invention.

20 For example, when the allergen is a cat allergen such as the Fel d 1 protein, then the MHC molecule may include DR13 or DR1 class II MHC, and a peptide that binds to DR13 and/or DR1 or any of its sub-types that may be used in a desensitisation procedure is that shown in SEQ. ID No. 3.

25 The peptides identified in such a manner, and those of use in the methods of the present invention may be used in desensitisation procedures that typically involve sequential administration of said peptide. Although the first administration of the peptide may induce a measurable or observable LPR, as has been described elsewhere the peptide or composition administered to the patient may be at a concentration that does not invoke

a measurable or observable LPR. Subsequent administration will lead to desensitisation of the patient. For example, if the peptide is that of SEQ. ID No. 3 (a fragment of the Fel d 1 allergen), then upon first administration of this peptide a LPR will be observed. Subsequent 5 administration of this peptide results in a weaker reaction or no reaction, the patient having been desensitised.

The invention also relates to the use of a peptide in desensitising a patient against an allergen, the peptide being identified by its capability to bind to 10 at least one MHC Class II molecule present in an individual and induce LPR in an individual who possesses the said MHC Class II molecule, wherein the patient also possesses the given MHC Class II molecule.

Peptides may be administered to a patient singly or in combination (for 15 example as a composition as defined above). Thus, the database according to the invention may be used to prepare a designer vaccine which may be used to desensitise a patient to a chosen allergen, on the basis of the patient's MHC Class II type. The MHC Class II type can be correlated to the known MHC Class II binding characteristics of the 20 peptides listed in the database, and the appropriate peptides selected and combined to form a designer vaccine. Similarly, the database may be used to design compositions (ie mixtures of peptides) which contain sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a late 25 phase response in an individual who possesses the said MHC Class II molecule, such that for at least 75% (preferably at least 80% or 85% or 90%) of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with the appropriate MHC Class II molecule.

Whilst it may be possible to design a vaccine which targets all or most of the epitopes on a particular antigen, this is unnecessary due to linked suppression of T-cells. Linked suppression is a phenomenon in which administration of a single epitope from a protein leads to the induction of a population of regulatory peptide-specific T lymphocytes which, by release of soluble factors such as TGF $\beta$  and/or IL-10, are able to suppress or modify responses of non-tolerant T cells specific for other epitopes within the same protein and in some models epitopes derived from other proteins ("bystander suppression") (Davies *et al* (1996) *J Immunol* 156:3602-7).

In transplantation models, such regulatory T cells have been demonstrated to be capable of inducing a similar phenotype in naive T cells. This has given rise to the term "infectious tolerance" (Qin *et al* (1993) *Science* 259:974-7) which may be a mechanism for effecting long-term hyporesponsiveness.

Linked suppression is thought to occur when peptide-specific regulatory T cells engage peptide/MHC complexes on the surface of the same or neighbouring APC as T cells specific for other epitopes. The latter may be responding to epitopes derived from the same molecule as the regulatory T cells or from a distinct molecule being processed by the same APC. This phenomenon allows desensitisation of patients to one or multiple allergens by the administration of a limited number of peptides.

Whilst it may be possible for the peptides or compositions according to the invention to be presented in raw form, it is preferable to present them as a pharmaceutical formulation. Thus, according to a further aspect, the present invention provides a pharmaceutical formulation comprising a peptide or composition according to the invention together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients. The carrier(s) must be 'acceptable' in

the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Typically, carriers for injection, and the final formulation, are sterile and pyrogen free.

5    The formulations include those suitable for oral (particularly inhaled), parenteral (including subcutaneous, transdermal, intradermal, intramuscular and intravenous and rectal) administration, although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented  
10   in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of the present invention as herein defined or a pharmacologically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients.

15   Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a  
20   non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Formulations for inhalation may be presented in any of the ways known to be effective eg metered dose inhalers.

25   Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening  
30   agents. The formulations may be presented in unit-dose or multi-dose

containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be 5 prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

10

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

15

The compounds of the invention may typically be administered intranasally, by inhalation, orally or *via* injection at a dose of from 0.0001 to 1 µg/kg per dose. Preferred are doses in the region of 10 to 150 µg per human patient, advantageously about 80 µg.

20

A further aspect of the invention provides a method of determining an initial dose of an immunotherapeutic peptide for desensitising a patient to a polypeptide allergen, which peptide is derived from the allergen and wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late 25 phase response in an individual who possesses the said MHC molecule, the method comprising (1) determining the dose which is able to generate an observable late phase response in a given proportion of individuals who possess the said MHC molecule and in whom the peptide is able to induce a late phase response and (2) selecting a lower dose which is incapable of 30 inducing an observable late phase response in substantially all individuals

who possess the said MHC molecule and in whom the peptide is able to induce a late phase response.

Preferably, the individuals who possess the said MHC molecules are  
5 appropriately sensitised; that is to say that the individuals have been sensitised previously to the allergen in question.

The initial dose which is administered to the patient to be desensitised is, as is described above, one which may not itself give rise to an observable  
10 LPR.

In step (1) of the method of determining an initial dose the given proportion of individuals may be any suitable proportion of, but not all, individuals as given. Typically, the proportion is 50% of individuals as  
15 given, but it may be, for example, 30% or 40% or 60% or 70% of individuals as given. In step (2), the lower dose may be the maximum dose that is incapable of inducing an observable late phase response in substantially all individuals who possess the said MHC molecules and in whom the peptide is able to induce a LPR.

20 Typically, but it will be appreciated that this will vary from peptide to peptide, the lower dose is between 10-fold and 100-fold lower than the dose which induces an observable LPR in 50% of suitable individuals (a suitable individual is one who is appropriately sensitised and has the  
25 appropriate MHC Class II molecule(s) to facilitate peptide reactivity).

The LPR may be any suitable LPR as herein disclosed. Suitably, late asthmatic reactions are determined in asthmatics, late nasal reactions in rhinitics and late phase skin reactions in all allergic individuals.

It is preferred if the LPR is a late cutaneous reaction.

The methods of the invention are particularly suited for use in connection with human patients. However, it will be appreciated that animals, particularly mammals, and more particularly domestic and farm animals such as dogs and cats, may suffer from allergies due to polypeptide allergens. The methods of the invention include methods in connection with such animals. Although the specification refers to MHC and HLA Class II molecules, equivalent molecules exist in mammals other than humans as is well known in the art.

The invention is further described, for the purpose of illustration only, in the following examples, which refer to the figures.

Figure 1. The three peptides comprising FC1P (solid circles; 80 $\mu$ g) or vehicle control (open circles) are injected intradermally at time zero on two separate days. Forced expiratory volume in 1 second (FEV1) is measured at intervals as a readout of lung function over a 24hr period. The use of rescue medication is indicated by arrows.

Figure 2. Repeated administration of FC1P leads to a reduced lung response. Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators.

Figure 3. Murine L cells expressing two DR13 variants, DRB1\*1301 and 1302 are incubated overnight with each of the three FC1P peptides, or a

control peptide, or medium alone. Cells are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent assay. L cells are then incubated for 48 hours with T cells from a T cell line raised to whole cat dander (and including the Fel d 1 protein).

5 Proliferation of the T cells is measured by their incorporation of the radiolabelled compound  $^3\text{H}$ -thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other peptides/control.

10 Figure 4. Human fibroblasts expressing the DR1 allele DRB1\*0101 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. In T cell proliferation assays, T cells demonstrate a statistically significant response to the DR1 expressing cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other peptides/control.

15

Figure 5. Human fibroblasts expressing the DR4 alleles DRB1\*0404 and DRB1\*0405 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. Figure 5 a) and b):

20 in T cell proliferation assays, DRB1\* 0408 responder cells demonstrate a statistically significant response to the DRB1\*0405 expressing cells and peptide FC1P2 (EQVAQYKALPVVLENA) but not to DRB1\* 0404 expressing cells and peptide FC1P2 or to the other peptides/control.

25 Figure 6. Human fibroblasts expressing the DR4 allele DRB1\*0405 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. In T cell proliferation assays, DRB1\* 0405 responder cells demonstrate a statistically significant response to the DRB1\*0405 expressing cells and peptide FC1P2 (EQVAQYKALPVVLENA) but not to the other peptides/control.

30

Figure 7. The T cell proliferation responses observed in Figures 3, 4 and 6 are confirmed by [IL-5] measurement in Figures 7 (a), 7(b) and 7 (c) respectively. As expected, these results show that IL-5 production  
5 correlates with T-cell proliferation.

Figure 8. Hypothetical protein and peptides (15mers) derived from overlapping by one residue.

10 Figure 9. Multiple overlapping peptides (MOP) from the cat allergen Fel d I. The three sequences within the box were insoluble in aqueous solution and as a result were excluded from the MOP preparation for clinical use.

15 Figure 10. An example of a LAR induced by the Fel d I MOP. The intradermal administration of 13 peptides which comprise MOP (solid circles; 2.5 µg, day 1) induce a fall in FEV1 of greater than 20% at 3 hours. Control day administration of 30BU cat dander extract does not induce a fall in FEV1 (open circles). A second administration of MOP  
20 (solid triangles; 2.5 µg, day 66) results in an attenuated fall in FEV1 which does not reach 20%. Arrows indicate the use of rescue medication ( $\beta_2$  agonists).

25 Figure 11. Changes in the cutaneous late phase response to whole allergen 6 hours after intradermal administration of whole cat dander extract before and after intradermal administration of MOP.

Figure 12. The 3 peptides comprising FCIP (open down triangles; 80 µg, Figures (a), (b) and (c)) were administered intradermally to cat allergic  
30 asthmatic subjects inducing a fall in FEV1 of greater than 20% compared

to a control day (open circles; 30BU whole cat dander extract, Figures 12(a), (b) and (c)). A second administration of FC1P within 6 weeks (closed down triangles; 80 µg, Figure 12(a)) demonstrated an attenuation of the response. Following administration of FC1P greater than one year 5 after the initial dose (closed up triangles; 80 µg, Figures 12(a), (b) and (c)), a fall in FEV1 of similar magnitude to the initial injection was observed. Arrows indicate the use of rescue medication ( $\beta_2$  agonists).

*Schedule of sequences for sequence listing:*

10 SEQ ID No 1: LFLTGT PDEY VEQVAQY (FC1P1)  
SEQ ID No 2: EQVAQY KALPVVLENA (FC1P2)  
SEQ ID No 3: KALPVVLENARILKNCV (FC1P3)  
SEQ ID No 4: Fel d I chain 1 in Figure 9  
SEQ ID No 5: Fel d 2 chain 2 in Figure 9

15

Other SEQ ID Nos. for peptides are shown on Figure 9.

**EXAMPLES**

20 **Experimental Techniques**

*Primary Proliferation Assays*

PBMCs are separated from whole blood by density gradient centrifugation according to standard methods. Cultures are established at  $2 \times 10^5$  cells per 25 well in flat bottomed 96 well plates with 3 concentrations each individual peptide, or an optimum concentration of cat dander cat allergen extract, medium (negative control) or PPD (positive control). Cells are cultured for 8 days (cat dander) and 6 days (all others) and pulsed with  $1\mu\text{Ci}$  tritiated thymidine. Cultures are harvested and counted after 8-16 hours.

30

*T Cell Clones*

PBMCs are cultured in 24 well plates with cat dander for 10 - 12 days, with the addition of approximately 10ng IL-2 on days 5 and 7, restimulated twice with irradiated autologous PBMCs and cat dander, and 5 the line expanded with Phytohaemagglutinin (PHA) and IL-2. Clones are established by limiting dilution and will subsequently be frozen for use at a later stage to determine changes in cytokine secretion.

Example 1: Preparation Of Allergen Peptides

10

The sequence of chain 1 of the cat allergen Fel d 1 is shown in Figure 9 (SEQ. ID. No. 4); chain 2 is also shown in Figure 9 (SEQ. ID. No. 5). Multiple overlapping peptides are designed around this sequence, as well as that of chain 2 of Fel d 1, as shown in Figure 9.

15

Example 2: Observation Of LAR In Patients On Peptide Administration

20

A single intradermal administration (80 $\mu$ g of each peptide) of a mixture containing three short peptides (Figure 9; (SEQ. ID Nos. 1, 2 or 3)) is given to 18 cat asthmatic individuals. 6 patients develop an isolated late asthmatic reaction as shown in Figure 1 wherein a greater than 20% fall in Forced Expiratory Volume in 1 second (FEV1 - a measure of lung function) is considered as a positive asthmatic effect. The results are 25 shown in Figure 1 where the three peptides comprising FC1P [solid circles; FC1P comprises FC1P1 (SEQ. ID. No. 1), FC1P2 (SEQ. ID. No. 2) and FC1P3 (SEQ. ID. No. 3)] or vehicle control (open circles) are injected intradermally at time zero on two separate days. FEV1 is measured at intervals as a readout of lung function over a 24hr period. 30 The use of rescue medication is indicated by arrows.

This result demonstrates that peptides capable of causing a LPR can be derived from a common allergen such as cat dander and tested for LAR production in cat asthmatic individuals.

5

Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators. As shown in Figure 2, none of the three develop a late asthmatic reaction to the second peptide administration indicating that the immune response to this peptide has been downregulated.

15 Example 3: Correlation between Tissue type and LAR

The 18 patients observed in Example 2 are MHC-typed using PCR, based upon the method of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Four of the 6 reactors express HLA-DR13 (a closely related family of MHC molecules) compared to 1 out of 12 of the non-reactors. These results indicate that one of the three peptides injected is capable of binding to a DR13 family member and thus stimulating peptide-specific T cells from the reactors.

25 In order to demonstrate that specific T cells have been activated, L cells which have been transfected with the human genes encoding two DR13 family members are obtained from Georgetown University Medical School, USA. (DR13 is a split of DR6). Murine L cells expressing two DR13 variants, DRB1\*1301 and 1302 are incubated overnight with each 30 of the three FC1P peptides, or a control peptide, or medium alone. Cells

00000000000000000000000000000000

are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent assay. L cells are then incubated for 48 hours with T cells from a T cell line raised from PBMCs isolated from a reactor patient as described above and stimulated weekly with whole cat dander (and including the Fel d 1 protein). Proliferation of the T cells is measured by their incorporation of the radiolabelled compound <sup>3</sup>H-thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 3.

10

A further experiment is performed with human fibroblasts expressing the DR1 variant DRB1\*0101. Cells are incubated overnight with each of the three FC1P peptides, or medium alone, washed, treated and incubated with T-cells as described above for the DR13 variants. T cells demonstrate a statistically significant response to the DR1 L cells and peptide FC1P3 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 4.

It is demonstrated that FC1P3 is capable of binding to both DR1 and DR13 MHC molecules and activating T cells, thereby inducing the isolated late asthmatic reaction shown in Figure 1. This result correlates extremely well with the tissue type data obtained from the patient population, wherein 4 out of six reactors are DR13 and two are DR1, compared with 1 out of 12 DR1 and 1 out of 12 DR13 non-reactors.

25

In a further series of experiments, patients reacting to FC1P are identified which express HLA-DR4 (DRB1\*0405 and 0408). The same experiments are conducted as set forth above for HLA-DR13 patients, using DRB1\* 0404 and 0405 L-cells (0408 cells are not available). The results are shown in Figure 5 and Figure 6.

The results indicate that patients expressing DRB1\* 0408 respond to FC1P2 presented by 0405 L cells but not 0404 L cells or to other peptides or controls. Likewise, patients expressing DRB1\* 0405 respond to 5 FC1P2 presented by 0405 L cells but not to other peptides or controls.

Figure 7 shows the IL-5 secretion levels for DR13(a), DR1(b) and DR4(c) HLA types which correlate with T cell proliferation data as expected.

10 **Example 4: FC1P3 induces LAR and desensitisation in tissue-typed patients**

Patients are selected on the basis of being allergic to cat dander, as in the previous examples. T-cell lines are prepared from each patient as 15 described above, and maintained with weekly stimulation with cat dander extract. The patients are tissue-typed, and patients possessing DR1 or DR13 variants selected.

In order to predict the ability of peptide FC1P3 to desensitise the patients 20 against cat dander, T-cell proliferation assays are performed using T-cells isolated from the patients as described and human fibroblasts or murine L-cells transfected with DR1 or DR13 alleles in the presence of FC1P3 according to Example 3. The T-cells are observed to proliferate, by the incorporation of  $^3\text{H}$ -thymidine, indicating that T-cells isolated from DR13 25 and DR1 possessing patients are responsive to stimulation with the FC1P3 peptide.

FC1P3 peptide is injected into patients which are DR1 and/or DR13 positive and in respect of whom a positive result has been obtained in the 30 T-cell proliferation assay. These patients experience a LAR response, as

measured by a 20% or greater fall in FEV1.

Patients who develop a late asthmatic reaction following administration of FC1P3 are challenged again with the same dose after a period of 2 weeks.

- 5 As in Example 2, no significant fall or a reduced fall in FEV1 is observed following the second challenge, indicating that the immune response to
- this peptide has been downregulated.

#### Example 5: MHC restriction mapping of Fel d 1

10

In order to prepare a database of Fel d 1 derived peptides characterised according to MHC type restriction, an *in vitro* study of MHC class II restriction mapping is performed using a panel of L cells, T-cell lines to whole cat allergen and the overlapping peptides from chain 1 and chain 2,

- 15 as described in Example 1. T cell lines with specificity for whole cat extract (which includes Fel d 1) are generated from the peripheral blood of subjects before peptide administration according to the procedures described above. Subjects are HLA-DR, DP and DQ typed, and, based on their expression, initially of DR alleles, transfected fibroblasts are
- 20 selected to assay T-cell stimulation by each of the peptides.

Where the required HLA type clone is not available, MHC genes are cloned directly from the patient's cells by PCR amplification and cloning, as described above. Cloned genes are subsequently expressed in murine

- 25 L-cells.

Cell lines (generous gifts from Prof. J.R. Lamb, University of Edinburgh, Prof. R.I. Lechler and Dr. G. Lombardi, ICSM, Hammersmith Hospital, Dr. C. Hurley and Dr. J.R. Richert, Georgetown University Medical

- 30 Center, Washington, USA) expressing the appropriate restriction element

are incubated with each individual Fel d 1 peptide as described in Example 3. Equivalent cell lines are generally available or may be readily made by transfecting appropriate genes expressing MHC Class II molecules. Following incubation in the cytostatic agent mitomycin C to prevent L cell division, cells are extensively washed and incubated with the T cell line. Proliferative responses are measured after 48 hours by addition of tritiated thymidine to all cultures for 8-16 hours. Peptides eliciting a proliferative response from the T cell line are thus restricted by the HLA allele expressed by the chosen L cell line.

10

Administration of peptides obtained from the database to patients possessing the HLA type in respect of which a proliferative response is seen in the above assay in the majority of cases results in a LAR, as expected, which is followed by desensitisation of the patient to cat dander 15 on subsequent administration of the peptides.

In this way an MHC class II restriction map of the Fel d 1 molecule is constructed such that the appropriate peptides for immunotherapy may subsequently be selected on an individual patient basis, solely by virtue of 20 that subject's HLA type.

Example 6: Identification of MHC-restricted peptides capable of inducing late phase reactions in individuals possessing the appropriate MHC molecule

25

(1) Overlapping peptides of 15 amino acid residues (range approx. 7-20) which are offset by one residue are chemically synthesised for example using FastMoc chemistry. An example of a hypothetical protein and the overlapping peptides (in this example 15mers) which may be 30 derived from it is given in figure 8.

(2) Each individual peptide is incubated with murine or human cells such as fibroblasts for example, which have been transfected with, or  
5 already express, the genes encoding a particular MHC molecule such as, for example DRA and DRB1\*0101. The concentration of peptide used for the incubation stage may vary from approximately 0.01mg/ml to 1mg/ml or more. An example is 200 $\mu$ g/ml. The incubation period may vary from approximately a few minutes to several hours. An example is 16 hours.

10

(3) Following incubation with peptide, the cells are washed several times (for example 3 times) in tissue culture medium (for example RPMI-1640 medium supplemented with 5% normal human AB serum, 2mM L-glutamine, 100microgram/ml streptomycin and 100U/ml penicillin).

15

(4) Cells are then incubated with mitomycin C (at approximately 50 $\mu$ g/ml) or another suitable cytostatic agent to prevent cell division. Cells are washed several times (for example 5 times) in culture medium and dispensed into 96 well tissue culture plates at a concentration of  
20 approximately  $3 \times 10^4$  cells per well for example.

(5) To these cells are added approximately  $1 \times 10^4$  cells of a T lymphocyte cell line which has been cultured in the presence of, and is reactive with, the protein from which the peptides in step (1) were derived. The MHC molecules expressed by the individual from which the T lymphocyte line was raised would usually include the MHC molecule expressed on the cells in step (2). Alternatively, the MHC molecules expressed by the individual from whom the T lymphocyte line was raised may differ from those expressed on the cells in step (2). Additionally, T  
25 lymphocytes from the same cell line are cultured on their own and also  
30

with the MHC-expressing cells described in stage (2) which have either not been incubated with a peptide, or have been incubated with an irrelevant peptide such as a peptide from another protein.

5 6) The cell mixture is cultured for approximately 2-3 days prior to the addition to each well of approximately 37MBq (1 $\mu$ Ci) of tritiated thymidine or similar for several hours (for example 6-16 hours).

10 7) Cultures are then harvested onto glass fibre filters and cellular proliferation (of the T lymphocytes), as correlated with uptake of tritiated thymidine into the DNA of the cells, is measured by liquid scintillation spectroscopy or a similar technique.

15 Peptides capable of binding to the relevant MHC molecules and inducing T cell activation are identified by the incorporation of the tritiated thymidine into the newly synthesised DNA of the activated T cells. When the DNA is analysed by liquid scintillation spectroscopy (or other suitable techniques) the radioactive label (tritium) generated counts per minute which correlate with the degree of T cell proliferation and thus activation.

20 Thus, MOPs derived from a polypeptide allergen are useful principally in the selection procedure for identifying the one or more useful peptides (which show MHC Class II restriction and which are able to give rise to a LPR in an individual who possesses the appropriate MHC Class II molecules) which may be used either individually or in combination as an immunotherapeutic agent.

25 The following is a list of known allergen sequences and database accession numbers (NCBI Entrez accession numbers). NCBI is the National Center for Biotechnology information and is a division of the US National

Institutes of Health. The NCBI web site, from which access to the database may be sought, [www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/). The allergens may be used as described above in order to identify MHC-restricted peptides capable of inducing LPR in individuals who possess a particular MHC molecule.

Allergen sequences and database accession numbers (NCBI Entrez accession numbers):

10 **House dust mite**

Dermatophagoides pteronyssinus

Der p 1

15 MKIVLAIASLLALSAVYARPSSIKTFEEYKKAFNKSYATFEDEEAAR  
KNFLESVKYVQSNGGAINHLSQLSLDEFKNRFLMSAEAFEHLKTQF  
DLNAETNACSGINGNAPAEIDLQRQMRTVTPIRMQGGCGSCWAFSGV  
AATESAYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQHN  
GVVQESYYRYVAREQSCRRPNAQRFGISNYCQIYPPNVNKIREALA  
QTHSAIAVIIGIKDLDARHYDGRTIIQRDNGYQPNYHAVNIVGYSN  
20 AQGVVDYWIVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL

Der p 2

25 MMYKILCLSLLVAAVARDQVDVKDCANHEIKKVLVPGCHGSEPCII  
HRGKPFQLEAVFEANQNTAKIEIKASIDGLEVDVPGIDPNACHY  
MKCPLVKGQQYDIKYTNVPKIAPKSENVVTVKVMGDDGVLAC  
AIATHAKIRD

Der p 3

30 MIIYNILIVLLLAIINTLANPILPASNATIVGGEKALAGECPYQISLQS  
SSHFCGGTILDEYWILTAAHCVAGQTASKLSIRYNSLKHSLGGEKIS

VAKIFAHEKYDSYQIDNDIALIKLKSPMKLNQKNAAVGLPAKGSD  
VKVGDQRVSGWGYLEEGSYSLPSELRRVDIAVVSRKECNELYSKA  
NAEVTDNMICGGDVANGGKDSCQGDSGGPVVDVKNNQVVGIVSW  
GYGCARKGYPGVYTRVGNFIDWIESKRSQ

5

Der p 4

KYXNPHFIGXRSVITXLME

Der p 5

10 MKFIIAFFVATLA VMTVSGEDKKHDYQNEFDPLL MERIHEQIKKGE  
LALFYLQE QINHFEEKPTKEMKDKIVAEMDTI IAMIDGVRGVL DRL  
MQRKDLDIFEQYNLEMAKKSGDILERDLKKEARVKKIEV

Der p 6

15 AIGXQPAAEAEAPFQISLMK

Der p 7

MMKLLLIAAAAFVA VSADPIHYDKITEEINKA VDEA VAAIEKSETFD  
PMKVPDHSDKFERHIGIDLKGELDMRNIQVRGLKQM KRG DAV  
20 KSEDGVVKAHLLVGVHDDVVSMEYDLAYKL GDLHPNTHVISDIQD  
FVVELSLEVSEEGNMTLSFEVRQFANVVNHIGGLSILDPIFAVLSD  
VLTAIFQDTVRAEMTKVLAPAFKKE LERN NQ

Der p9

25 IVGGSNASPGDAVYQIAL

Dermatophagoides farinae

Der f 1

30 MKFVLAIASLLVLT VYARPASIKTFEFKKAFNKNYATVEEEEVARK

NFLESLKYVEANKGAINHLSDSLDEFKNRYLMSAEAFEQLKTQFD  
LNAETSACRINSVNVPSEDLRSLRTVTPIRMQGGCGSCWAFSGVA  
ATESAYLAYRNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQQNG  
VVEERSYPYVAREQRCCRPNSQHYGISNYCQIYPPDVKQIREALTQT  
5 HTAIAVIIGIKDLRAFQHYDGRTIIQHDNGYQPNEYAVNIVGYGSTQ  
GDDYWIVRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVVIM

## Der f 2

MISKILCLSLLVAAVVADQVDVKDCANNEIKVMVDGCHGSDPCII  
10 HRGKPFTLEALFDANQNTKTAKIEIKASLDGLEIDVPGIDTNACHFM  
KCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKLIGDNGVLACAIA  
THGKIRD

## Der f 3

15 MMILTIVVLLAANILATPILPSSPNATIVGGVKAQAGDCPYQISLQSS  
SHFCGGSILDEYWILTAAHCVNGQSAKKLSIRYNTLKHASGGEKIQV  
AEIYQHENYDSMTIDNDVALIKLKTPMTLDQTNAKPVPLPAQGSDV  
KVGDKIRVSGWGYLQEGLPSLQSELQRVDIDVV~~SREQCDQLYSKAG~~  
ADVSENMICGGDVANGGVDSQGDGGPVVDVATKQIVGIVSWGY  
20 GCARKGYPGVYTRVGNFVDWIESKRSQ

## Der f 4

AVGGQDADLAEAPFQISLLK

## 25 Der f 7

MMKFLLIAAVAFVAVSADPIHYDKITEEINKAIDDAIAAIEQSETIDP  
MKVPDHADKFERHVGIVDFKGELAMRNIEARGLKQMKRQGDANV  
KGEEGIVKAHLLIGVHDDIVSMEYDLAYKLGDLHPTTHVISDIQDF  
VVALSLEISDEGNITMTSFEVRQFANVVNHIGGLSILDPIFGVLSDVL  
30 TAIFQDTVRKEMTKVLAPAFKRELEKN

Additional mite allergen sequences (NCBI entrez accession):

1170095; 1359436; 2440053; 666007; 487661; 1545803; 84702; 84699;  
5 625532; 404370; 1091577; 1460058; 7413; 9072; 387592.

**Cat**

**Felis sequences**

10 1082946 Fel dI chain 2 precursor - cat

MRGALLVLALLVTQALGVKMAETCPIFYDVFFAVANGNELLDSL  
LTKVNATEPERTAMKKIQDCYVENGLISRVDGLVMTTISSSKDCM  
GEAVQNTVEDLKLNTLGR

15 1082945 Fel dI chain 1 short form - cat

MLDAALPPCPTVAATADCEICPAVKRDVDLFLTGTGTPDEYVEQVAQ  
YKALPVVLENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC

1082944 Fel dI chain 1 long form precursor - cat

20 MKGARVLVLLWAALLLIWGGNCEICPAVKRDVDLFLTGTGTPDEYVE  
QVAQYKALPVVLENARILKNCVDAKMTEEDKENALSLLDKIYTSPL  
C

Additional Felis sequences (NCBI entrez accession):

25

539716; 539715; 423193; 423192; 423191; 423190; 1364213; 1364212;  
395407; 163827; 163823; 163825; 1169665; 232086; 1169666.

**Latex**

30 Hevea sequences:

Hev b 1

MAEDEDNQQGQGEGLKYLGFVQDAATYAVTTFNSNVYLFAKDKSG  
PLQPGVDIIEGPVKNVAVPLYNRFSYIPNGALKFVDSTVVASVTIIDR  
5 SLPPIVKDASIQQVVAIRAAPEAARSLASSLPGQTKILAKVFYGEN

Hev b 3

MAEEVEEERLKYLDFVRAAGVYAVDSFSTLYLYAKDISGPLKPGV  
DTIENVVKTVVTPVYYIPLEAVKFVDKTVDVSVTSDLGVVPPVIKQ  
10 VSAQTYSVAQDAPRIVLDVASSVFNTGVQEGAKALYANLEPKAEQ  
YAVITWRALNKLPLVPQVANVVVPTAVYFSEKYNDVVRGTTEQGY  
RVSSYLPPLLPTEKITKVFGDEAS

Additional Hevea sequences (NCBI entrez accession):

15 3319923; 3319921; 3087805; 1493836; 1480457; 1223884; 3452147;  
3451147; 1916805; 232267; 123335; 2501578; 3319662; 3288200;  
1942537; 2392631; 2392630; 1421554; 1311006; 494093; 3183706;  
3172534; 283243; 1170248; 1708278; 1706547; 464775; 266892;  
231586; 123337; 116359; 123062; 2213877; 542013; 2144920; 1070656;  
20 2129914; 2129913; 2129912; 100135; 82026; 1076559; 82028; 82027;  
282933; 280399; 100138; 1086972; 108697; 1086976; 1086978;  
1086978; 1086976; 1086974; 1086972; 913758; 913757; 913756;  
234388; 1092500; 228691; 1177405; 18839; 18837; 18835; 18833;  
18831; 1209317; 1184668; 168217; 168215; 168213; 168211; 168209;  
25 348137.

Rye grass

Lolium sequences:

30 126385 Lol p 1

MASSSVLLVVALFAVFGLSAHGIKVPPGPNITAELYGDKWLDAKS  
 TWYGKPTGAGPKDNGGACGYKNVDKAPFNGMTGCGNTPIFKDGR  
 GCGSCFEIKCTKPESCSEAVTVTITDDNEEPIAPYHFDSLGHAFGS  
 MAKKGEEQNVRSAGELELQFRRVKCKYPDDTKPTFHVEKASNPNY  
 5 LAILVKYVDGDDGVVAVDIKEKGKDKWIELKESWGA VWRIDTPDK  
 LTGPFTVRYTTEGGTKSEFEDVIPEGWKADTSYSAK

126386 Lol p 2a

AAPVEFTVEKGSDEKNLALSINKYNKEGDSMAEVELKEHGSNEWLA  
 10 LKKNGDGVWEIKSDKPLKGPFNFRFVSEKGMRVFDDVVVPADFKV  
 GTTYKPE

126387 Lol p 3

TKVDLTVEKGSDAKTLVLNIKYTRPGDTLAEVELRQHGSEEWEPM  
 15 TKKGNLWEVKSAKPLTGPBMNFRFLSKGGMKNVFDEVIPTAFTVGK  
 TYTPEYN

2498581 Lol p 5a

MAVQKYTVALFLRRGPRGGPGRSYAADAGYTPAAAATPATPAATP  
 20 AGGWREGDDRRAEAAGGRQRLASRQPWPPLPTPLRRRTSSRSSLRPPS  
 PSPPRASSPTSAAKAPGLIPKLDTAYDVAYKAAEAHPRGQVRRRLH  
 CPHRSLRVIAGALEVHAVKPATEEVLAAKIPTGELQIVDKIDAIFI  
 AATAANAAPTNDKFTVFESAFNKALNECTGGAMRPTSSPPSRPRS  
 SRPTPPPSPAAPEVKYAVFEAALT KAITAMTQAQKAGKPAAAATA  
 25 AATVATAAAATAAVLPPLLVVQLISLLIYY

2498582 Lol p 5b

MAVQKHTVALFLAVALVAGPAASYAADAGYAPATPATPAAPATA  
 ATPATPATPATPAAVPSGKATTEEQKLIIEKINAGFKA VAAA AVVP  
 30 PADKYKTFVETFGTATNKA FVEGLASGYADQSKNQLTSKLDAAALK

LAYEAAQGATPEAKYDAYVATLTEALRVIAGTLEVHAVKPAEEV  
KVGAIPAAEVQLIDKVDAAYRTAATAANAAPANDKFTVFENTFNN  
AIKVSLGAAYDSYKFIPLVAAVKQAYAAKQATAPEVKYTVSETAL  
KKAVTAMSEAEKEATPAAAATATPTPAAATATATPAAAYATATPA  
5 AATATATPAAATATPAAAGGYKV

455288 Lol p isoform 9

MAVQKHTVALFLAVALVAGPAASYAADAGYAPATPATPAAPATA  
ATPATPATPATPAAVPSGKATTEEQKLIEKINAGFKAAVAAAAVVP  
10 PADKYKTFVETFGTATNKAFVEGLASGYADQSKNQLTSKLDAAALK  
LAYEAAQGATPEAKYDAYVATLTEALRVIAGTLEVHAVKPAEEV  
KVGAIPAAEVQLIDKVDAAYRTAATAANAAPANDKFTVFENTFNN  
AIKVSLGAAYDSYKFIPLVAAVKQAYAAKQATAPEVKYTVSETAL  
KKAVTAMSEAEKEATPAAAATATPTPAAATATATPAAAYATATPA  
15 AATATATPAAATATPAAAGGYKV

1582249 Lol p 11

DKGPGFVVTGRVYCDPCRAGFETNVSHNVEGATVAVDCRPFDGG  
ESKLKAEATTDKDGWYKIEIDQDHQEEICEVVLAKSPDKSCSEIEEF  
20 RDRARVPLTSNXGIKQQGIRYANPIAFFRKEPLKECGGILQAY

Additional *Lolium* sequences (NCBI entrez accession):

135480; 417103; 687261; 687259; 1771355; 2388662; 631955; 542131;  
25 542130; 542129; 100636; 626029; 542132; 320616; 320615; 320614;  
100638; 100634; 82450; 626028; 100639; 283345; 542133; 1771353;  
1763163; 1040877; 1040875; 250525; 551047; 515377; 510911; 939932;  
439950; 2718; 168316; 168314; 485371; 2388664; 2832717; 2828273;  
548867.

**Olive tree****Olive sequences**

416610 Ole e 1

5 EDIPQPPVSQFHIQGQVYCDTCRAGFITELSEFIPGASLRLQCKDKEN  
GDVTFTEVGYTRAEGLYSMLVE  
RDHKNEFCEITLISSGRKDCNEIPTEGWAKPSLKFKLNTVNGTTRTV  
NPLGFFKKEALPKCAQVYNKLM  
YPPNM

10

**Parietaria****Parietaria sequences:**

2497750 Par j P2

15 MRTVSMAALVVIAAALAWTSSAEPAPAPAPGEEACGKVVQDIMP  
LHFVKGEEKEPSKECCSGTKKLSEEVKTTEQKREACKCIVRATKGIS  
GIKNELVAEVPKKCDIKTLPPITADFDCKIYSTIFRGYY

1352506 Par j P5

20 MVRALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACE  
CIQTAMKTYSDIDGKLVSEVPKHCGIVDSKLPPIDVNMDCKTVGVV  
PRQPQLPVSLRHGPVTGPSDPAHKARLERPQIRVPPPAPEKA

1532056 Par j P8

25 MRTVSMAALVVIAAALAWTSSAELASAPAPGEGPCGKVVHHIMPC  
LKFKVKGEEKEPSKSCCSGTTKLSSEEVKTTEQKREACKCIVAATKGIS  
GIKNELVAEVPKKCGITTLPPITADFDCKIESTIFRGYY

1532058 Par j P9

30 MRTVSAPSVALVVIAGLAWSLASVAPPAPAPGSEETCGTVVR

ALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGLQRVHACECIQT  
 AMKTYSDIDGKLVSEVPKHCGIVDSLPPIDVNMDCKTLGVVPRQP  
 QLPVSLRHGPVTGPSDPAHKARLERPQIRVPPPapeka

5 2497749 Par j P9

MRTVSARSSVALVVIVAAVLVWTSSASVAPAPAPAGSEETCGTVVGA  
 - LMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACECIQTA  
 MKTYSDIDGKLVSEVPKHCGIVDSLPPIDVNMDCKTLGVLHYKG  
 N

10

1086003 Par j 1

MVRALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACE  
 CIQTAMKTYSDIDGKLVSEVPKHCGIVDSLPPIDVNMDCKTVGVV  
 PRQPQLPVSLRHGPVTGPSRSRPPTKHGWRDPRLEFRPPHRKKPNP  
 15 AFSTLG

Additional Parietaria sequences (NCBI entrez accession):

543659; 1836011; 1836010; 1311513; 1311512; 1311511; 1311510;  
 20 1311509; 240971.

**Timothy grass**

Phleum sequences:

25 Phl p 1

MASSSVLLVVVLFAVFLGSAYGIPKVPPGPNTATYGDKWLDAKS  
 TWYGKPTGAGPKDNGGACGYKDVKPPFSGMTGCGNTPIFKSGRG  
 CGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDSLGHAFGAM  
 AKKGDEQKLRSAQELELQFRRVKCKYPEGTKVTFHVEKGSNPNYL  
 30 ALLVKYVNGDGDVVAVDIKEKGKDKWIELKESWGAIWRIDTPDKL

0964013410262600

TGPFTVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Phl p 1

MASSSVLLVVALFAVFLGSAHGIPKVPPGPNITATYGDKWLDAKS  
5 TWYGKPTAACPKDNGGACGYKDVKPPSGMTGCGNTPIFKSGRG  
CGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAAHFDSLGSIAFGSM  
AKKGDEQKLRSAGEVEIQFRRVKCKYPEGTKVTFHVEKGSNPNYL  
ALLVKFSGDGDVVAVDIKEKGKDKWIALKESWGAIWRIDTPEVLK  
GPFTVRYTTEGGTKARAKDVIPEGWKADTA YESK

10

Phlp 2

MSMASSSSSSLLAMA VLAALFAGAWCVPKVTFTVEKGSNEKHLAV  
LVKYEGDTMAEVELREHGSDEWVAMTKGEVVWTFDSEEPLQGP  
FNFRFLTEKGMKNVFDDVVPEKYTIGATYAPEE

15

Phl p 5

ADLGYGGPATPAAPAEAAPAGKATTEEQKLIEKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSSKAALTS  
KLDAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV  
20 KPAAEEVKVIPAGELQVIEKVDSAFKVAATAANAAPANDKFTVFEA  
AFNNAIKASTGGA YESYKFIPALEAAVKQAYAATVATAPEVKYTGF  
ETALKKAFTAMSEAQKAAKPATEATATATAAVGAATGAATAATG  
GYKV

25 Phl p 5

ADLGYGGPATPAAPAEAAPAGKATTEEQKLIEKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSSKAALTS  
KLDAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV  
KPAAEEVKVIPAGELQVIEKVDSAFKVAATAANAAPANDKFTVFEA  
30 AFNNAIKASTGGA YESYKFIPALEAAVKQAYAATVATAPEVKYTGF

ETALKKAITAMSEAQKAAKPATEATATATAAVGAATGAATAATGG  
YKV

Phl p 5b

5    AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQ  
KLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSSKAAAACKAPG  
LVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEV  
HAVKPVTEEPGMAKIPAGELOIIDKIDAACKVAATAAATAPADDKF  
TVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPQV  
10    KYAVFEAALT KAITAMSEVQKVSQPATGAATVAAGAATTAAGAAS  
GAATVAAGGYKV

Phl p 5a

ADLGYGPATPAAPAAGYTPATPAAPAGADAAGKATTEEQKLIEKIN  
15    AGFKAALAGAGVQPADKYRTFVATFGPASNKAFAEGLSGEPKGAA  
ESSSKAALTSKLDAAYKLA YKTAEGATPEAKYDAYVATLSEALRII  
AGTLEVHAVKPAAEEVKVIPAGELOQVIEKVDAAFKVAATAANAAP  
ANDKFTVFEAAFNDEIKASTGGAYESYKFIPALEAAVKQAYAATVA  
TAPEVKYTVFETALKKAITAMSEAQKAAKPAAAATATATAAVGAA  
20    TGAATAATGGYKV

Phl p 5

MAVQKYTVALFLAVALVAGPAASYAADAGYAPATPAAAGAEAGK  
ATTEEQKLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSKAA  
25    TAKAPGLVPKLDAAYSVSYKAAVGATPEAKFDSFVASLTEALRIVIA  
GALEVHAVKPVTEEPGMAKIPAGELOIIDKIDAACKVAATAAATAP  
ADTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAP  
QVKYAVFEAALT KAITAMSEVQKVSQPATGAATVAAGAATTAAG  
AASGAATVAAGGYKV

Phl p 5

MAVQKYTVALFLAVALVAGPAASYAADAGYAPATPAAAGAEAGK  
ATTEEQKLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSKAA  
TAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIA  
5 GALEVHAVKPVTEDPAWPKIPAGELOIIDKIDAASFKAATAAAATAP  
ADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATV  
AAPQVKYAVFEAALTCAITAMSEVQKVSPATGAATVAAGAATT  
ATGAASGAATVAAGGYKV

10 Phl p 5

ADAGYAPATPAAAGAEAGKATTEEQKLIEDINVGFKAAVAAAASV  
PAADKFKTFEAAFTSSKAAATAKAPGLVPKLDAAYSVAYKAAVGA  
TPEAKFDSFVASLTEALRVIAAGALEVHAVKPVTEEPGMAKIPAGELO  
QIIDKIDAASFKAATAAAATAPADDKFTVFEAAFNKAIKESTGGAYD  
15 TYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTCAITAMSEV  
QKVSPATGAATVAAGAATTAAAGAASGAATVAAGGYKV

Phl p 5

SVKRSNGSAEVHRGA VPRRGPRGGPGRSYAADAGYAPATPAAAGA  
20 EAGKATTEEQKLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSS  
SKAATAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEA  
LRVIAGALEVHAVKPVTEEPGMAKIPAGELOIIDKIDAASFKAATAA  
ATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYA  
ATVAAAPQVKYAVFEAALTCAITAMSEVQKVSPATGAATVAAGA  
25 ATTAAGAASGAATVAAGGYKV

Phl p 5

MAVHQYTVALFLAVALVAGPAGSYAADLGYGPATPAAPAAGYTP  
ATPAAPAGAEPAGKATTEEQKLIEKINAGFKAALAAAAGVPPADKY  
30 RTFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSKLDAAVKLA

05646224 02 05000

YKTAEGATPEAKYDAYVATVSEALRIIAGTLEVHAVKPAEEEVKVI  
PAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEEAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAIT  
AMSEAQKAAKPAAAATATAAVGAATGAATAATGGYKV

5

Philip 5

ADLGYGGPATPAAPAEAAAGKATTEEQKLIEKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSSKAALTS  
KLDAAYKLAJKTAEGATPEAKYDAYVATLSEALRIAGTLEVHAV  
10 KPAAEVKVIPAGELVIEKVDSAFKVAATAANAAPANDKFTVFEA  
AFNNAIKASTGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVF  
ETALKKAFTAMSEAQKAAKPATEATATATAAVGAATGAATAATG  
GYKV

15 Phl p5b

AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQ.  
KLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSSKAAAAKAPG  
LVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEV  
HAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKF  
20 TVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPQV  
KYAVFEAALTKAITAMSEVQKVSPATGAATVAAGAATTAAGAAS  
GAATVAAGGYKV

Phl p5a

25 ADLGYGPATPAAPAAGYTPATPAAPAGADAAGKATTEEQKLIEKIN  
AGFKAALAGAGVQPADKYRTFVATFGPASNKAFAEGLSGEPKGAA  
ESSSKAALTSLDAAYKLAYKTAEGATPEAKYDAYVATLSEALRII  
AGTLEVHAVKPAAEEVKVIPAGELQVIEKVDAAFKVAATAANAAP  
ANDKFTVFEAAFNDEIKASTGGA YESYKFIPALEAAVKQAYAATVA  
30 TAPEVKYTVFETALKAITAMSEAQKAAKPAAAATATATAAVGAA

TGAATAATGGYKV

Phl p 5

AVPRRGPRGGPGRSYAADAGYAPATPAAAGAEAGKATTEEQKLIE  
5 DINVGFKAAVAAAASVPAGDKFKTFEAFAFTSSSKAATAKAPGLVPK  
LDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVK  
PVTEEPGMAKIPAGELOIIDKIDAALKVAATAAATAPADDKFTVFE  
AAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPQVKYA  
VFEAALT KAITAMSEVQKVSPATGAATVAAGAATTATGAASGAA  
10 TVAAGGYKV

Phl p 5b

MAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLI  
EDINVGFKAAVAARQRPAADKFKTFEAASPRHPRPLRQGAGLVPKL  
15 DAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKP  
VTEEPGMAKIPAGELOIIDKIDAALKVAATAAATAPADDKFTVFEA  
AFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAEEVKYAV  
FEAALT KAITAMSEVQKVSPATGAATVAAGAATTAAGAASGAAT  
VAAGGYKV

20

Phl p 5

MAVHQYTVALFLAVALVAGPAASYAADLGYGPATPAAPAAGYTP  
ATPAAPAEAAPAGKATTEEQKLIEKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSKLDAYKL  
25 AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAEEVKV  
IPAGELOVIEKVDAAFKVAATAANAAPANDKFTVFEAAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAIT  
AMSEAQKAAKPAAAATATAAVGAATGAATAATGGYKV

30 Phl p 5

EAPAGKATTEEQKLIKEKINAGFKAALARRLQPADKYRTFVATFGPA  
SNKAFAEGLSGEPKGAAESSSKAALTSKLDAAYKLA YKTAEGATPE  
AKYDAYVATLSEALRIIAGTLEVHAVKPAAEVVKVIPAAELQVIEKV  
DAAFKVAATAANAAPANDKFTVFEAAFNDEIKASTGGAYESYKFIP  
5 ALEAAVKQAYAATVATAPEVKYTGFETALKKAITAMSEAQKAACP  
PPLPPPQPPPLAATGAATAATGGYKV

## Phl p 5

MAVHQYTVALFLAVALVAGPAASYAADLGYGPATPAAPAAGYTP  
10 ATPAAPAEAAPAGKATTEEQKLIKEKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSKLDAAYKL  
AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAAEVVK  
IPAGELOVIEKVDAAFKVAATAANAAPANDKFTVFEAAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTGFETALKKAIT  
15 AMSEAQKAACPAAAATATAAVGAATGAATAATGGYKV

## Phl p 5b

MAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLI  
EDINVGFKAAVAARQRPAADKFKTFEAASPRHPRPLRQGAGLVPKL  
20 DAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKP  
VTEEPGMAKIPAGELOIIDKIDAALKVAATAAATAPADDKFTVFEA  
AFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAAEVKYAV  
FEAALT KAITAMSEVQKVSQPATGAATVAAGAATTAAGAASGAAT  
VAAGGYKV

25

## Phl p 5a

ADLGYGPATPAAPAAGYTPATPAAAPAGADAAGKATTEEQKLIKEKIN  
AGFKAALAGAGVQPADKYRTFVATFGPASNKAFAEGLSGEPKGAA  
ESSSKAALTSKLDAAYKLA YKTAEGATPEAKYDAYVATLSEALRII  
30 AGTLEVHAVKPAAEVVKVIPAGELOVIEKVDAAFKVAATAANAAP

ANDKFTVFEAAFNDEIKASTGGAYESYKFIPALEAAVKQAYAATVA  
TAPEVKYTVFETALKAITAMSEAQKAAKPPPLPPPQPPPLAATGA  
ATAATGGYKV

5 Phl p 5

MAVHQYTVALFLAVALVAGPAASYAADLGYGPATPAAPAAGYTP  
ATPAAPAEAAPAGKATTEEQKLIEKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSKLDAA~~YKL~~  
AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAAEEVKV  
10 IPAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEAAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKAIT  
AMSEAQKAAKPAAAATATATAAVGAATGAATAATGGYKV

Phl p 6

15 MAAHKFMVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDVNA  
SFRAAMATTANVPPADKYKTFEAAFTVSSKRLADAVSKAPQLVP  
KLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALRIIAGTPEVHAV  
KPGA

20 Phl p 6

SKAPQLVPKLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALHIIAG  
TPEVHAVKPGA

Phl p 6

25 ADKYKTFEAAFTVSSKRLADAVSKAPQLVPKLDEVYNAAYNAAD  
HAAPEDKYEAFVLHFSEALHIIAGTPEVHAVKPGA

Phl p 6

TEEQKLIEDVNASFRAAMATTANVPPADKYKTLEAAFTVSSKRNLADAVSKAPQLVPKLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALRIIAGTPEVHAVKPG

5

Phl p 6

MAAHKFMVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDINASFRAAMATTANVPPADKYKTFEAAFTVSSKRNLADAVSKAPQLVPKLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALHIIAGTPEVHAVK

10 PGA

Phl p 6

MVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDVNASFRAAMA  
TTANVPPADKYKTFEAAFTVSSKRNLADAVSKAPQLVPKLDEVYN  
15 AAYNAADHAAPEDKYEAFVLHFSEALRIIAGTPEVHAVKPG

Phl p 7

MADDMERIFKRFDTNGDGKISLSELTDALRTLGSSTADEVQRMMAEIDTDGDFIDFNEFISFCNANPGLMKDVAKVF

20

Phl p 11

MSWQTYVDEHLMCEIEGHHLASAAILGHGDGTWQAQSADFPQFKPE  
EITGIMKDFDEPGHLAPTGMFVAGAKYMIQGEPEGRVIRGKKGAG  
GITIKKTGQALVVGIYDEPMTPGQCNCMVVERLGDYLVEQGM

25

Additional Phleum sequences (NCBI entrez accession):

458878; 548863; 2529314; 2529308; 2415702; 2415700; 2415698;

542168; 542167; 626037; 542169; 541814; 542171; 253337; 253336;

30 453976; 439960 .

09564004234 - 02705800

Wasp (and related)

Vespa sequences:

5 465054 ALLERGEN VES V 5

MEISGLVYLIIIVTHIDLPYKGANNYCKIKCLKGGVHTACKYGSLKPN  
CGNKVVVSYGLTKQEKKDILKEHNDFRQKIARGLETRGNPGPQPPA  
KNMKNLVWNDELAYVAQVWANQCQYGHDTCRDVAKYQVGQNV  
ALTGSTAAKYDDPVKLVKMWEDEVKDYNPKKKFSGNDFLKTGHY  
10 TQMWWANTKEVGCGSIKYIQEKKWHLVCNYGPSGNFMNEELY  
QTK

1709545 ALLERGEN VES M 1

GPKCPFNSDTVIIIETRENNRDLYTLQTLQNHPEFKKKTITRPVVF  
15 ITHGFTSSASEKNFINLAKALVDKDNYMVISIDWQTAACTNEYPGL  
KYAYYPTAASNTRLVGQYIATITQKLVKDYKISMANIRLIGHSLGAH  
VSGFAGKRVQELKLGKYSEIIGLDPARPSFDSNHCSERLCETDAEYV  
QIIHTSNYLGTEKILGTVDFYMNNGKNNPQGCGRFFSEVCSHTRAVIY  
MAECIKHECCLIGIPRSKSSQPISRCTKQECVCVGLNAKKYPSRGSFY  
20 VPVESTAPFCNNKGKII

1352699 ALLERGEN VES V 1

MEENMNLKYLLLTVYFVQLNCCYGHGDPLSYELDRGPKCPFNSD  
TVSIIIETRENNRDLYTLQTLQNHPEFKKKTITRPVVFITHGFTSSAS  
25 ETNFINLAKALVDKDNYMVISIDWQTAACTNEAAGLKLYYPTAA  
RNTRLVGQYIATITQKLVKHYKISMANIRLIGHSLGAHASGFAGKKV  
QELKLGKYSEIIGLDPARPSFDSNHCSERLCETDAEYVQIIHTSNYLG  
TEKTLGTVDFYMNNGKNQPGCGRFFSEVCSHSRAVIYMAECIKHE  
CCLIGIPKSQSKSSQPISRCTKQECVCVGLNAKKYPSRGSFYVPVESTAP  
30 FCNNKGKII

1346323 ALLERGEN VES V 2  
SERPKRVFNIYWNVPTFMCHQYDLYFDEVTNFNIKRNSKDDFQGD  
KIAIFYDPGEFPALLSLKDGYKKRNGGPQEGNITIHLQKFIENLD  
5 KIYPNRNFSGIGVIDFERWRPIFRQNWGNMKIHKNFSIDLVRNEHPT  
WNKKMIELEASKRFEKYARFFMEETLKLAKKTRKQADWGYYGYP  
YCFNMSPNNLVPECDVTAMHENDKMSWLFNNQNVLPSVYVRQE  
LTPDQRIGLVQGRVKEAVRISNNLKHSPKVLSYWWYVYQDETNTF  
LTETDVKKTFQEIVINGGDIIWGSSDVNSLSKCKRLQDYLLTVLG  
10 PIAINVTEAVN

549194 ALLERGEN VES VI  
5KVNYCKIKCLKGGVHTACKYGTSTKPNCGKMKVVKAYGLTEAEK  
QEILKVHNDFRQKVAKGLETRGNPGPQPPAKNMNNLVWNDELANI  
15 AQVWASQCNYGHDTCKDTEKYPVGQNIKRSTTAALFDSPGKLVK  
MWENEVKDFNPNIEWSKNNLKKTGHYTQMVWAKTKEIGCGSVKY  
VKDEWYTHYLVCNYGPSGNFRNEKLYEKK

Additional vespula sequences (NCBI entrez accession):  
20 549193; 549192; 549191; 549190; 549189; 117414; 126761; 69576;  
625255; 627189; 627188; 627187; 482382; 112561; 627186; 627185;  
1923233; 897645; 897647; 745570; 225764; 162551.

Tree allergen sequences (mainly birch) sequences:  
25  
114922 Bet v 1  
MGVFNYETETTSVIPAARLFKAFLDGDNLFPKVAPQAISSVENIEG  
NGGPGTIKKISFPEGFPFKYVKDRVDEVDTNFKYNYSVIEGGPIGD  
TLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMG  
30 ETLLRAVESYLLAHSDAYN

130975 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSFPQFK  
PQEITGIMKDFEEPGLAPGLHLGGIKYMIQGEAGAVIRGKKGSG  
5 GITIKKTGQALVFGIYEEPVTGQCNCMVVERLGDYLIDQGL

1168696 Bet v 3

MPCSTEAMEKAGHGHASTPRKRSLSNSSFRLRSESLNTLRLRRIFDL  
FDKNSDGIITVDELSRALNLLGETDLSELESTVKSFTREGNIGLQFE  
10 DFISLHQSLNDSYFAYGGEDEDNEEDMRKSILSQEEADSFGGFKV  
FDEDGDGYISARELQMVLGKLGSEGSEIDRVEKMIVSVDSNRDGR  
VDFFEFKDMMRSLVRSS

809536 Bet v 4

15 MADDHPQDKAERERIFKRFDANGDGKISAAELGEALKTLGSITPDE  
VKHMMAEIDTDGDGFISFQEFTDFGRANRGLLKDVAKIF

543675 Que a I - Quercus alba = oak trees (fragment)

GVFTXESQETSVIAPAXLFKALFL

20 543509 Car b I - Carpinus betulus = hornbeam trees (fragment)  
GVFNYEAETPSVIPAARLFKSYVLDGDKLIPKVAPQAIXK

543491 Aln g I - Alnus glutinosa = alder trees (fragment)

25 GVFNYEAETPSVIPAARLFKAFLDGDKLLPKVAPEAVSSVENI

1204056 Rubisco

VQCMQVWPPLGLKKFETLSYLPPLSSEQLAKEVDYLLRKNLIPCLE  
FELEHGFVYREHNRSPGYYDGRYWTMWKLPMFGCNDSSQLKEL  
30 EECKKAYPSAFIRIIGFDDK

Additional tree allergen sequences (NCBI entrez accession number):

131919; 128193; 585564; 1942360; 2554672; 2392209; 2414158;  
5 1321728; 1321726; 1321724; 1321722; 1321720; 1321718; 1321716;  
1321714; 1321712; 3015520; 2935416; 464576; 1705843; 1168701;  
1168710; 1168709; 1168708; 1168707; 1168706; 1168705; 1168704;  
1168703; 1168702; 1842188; 2564228; 2564226; 2564224; 2564222;  
2564220; 2051993; 1813891; 1536889; 534910; 534900; 534898;  
10 1340000; 1339998; 2149808; 66207; 2129477; 1076249; 1076247;  
629480; 481805; 81443; 1361968; 1361967; 1361966; 1361965;  
1361964; 1361963; 1361962; 1361961; 1361960; 1361959; 320546;  
629483 ; 629482; 629481; 541804; 320545; 81444; 541814;; 629484;  
474911; 452742; 1834387; 298737; 298736; 1584322; 1584321; 584320;  
15 1542873; 1542871; 1542869; 1542867; 1542865; 1542863; 1542861;  
1542859; 1542857; 1483232; 1483230; 1483228; 558561; 551640;  
488605; 452746; 452744; 452740; 452738; 452736; 452734; 452732;  
452730; 452728; 450885; 17938; 17927; 17925; 17921; 297538; 510951;  
289331; 289329; 166953 .

20

**Peanut**

Peanut sequences

1168391 Ara h 1  
25 MRGRVSPLMLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSC  
QQEPDDLKQKACESRCTKLEYDPRCVYDPRGHTGTTNQRSPPGER  
TRGRQPGDYDDDRRQPRREEGGRWGPAGPREREREEDWRQPRED  
WRRPSHQQPRKIRPEGREGEQEWTGPGSHVREETSRRNNPFYFPSRR  
FSTRYGNQNNGRIRVLQRFDQRSRQFQLQNHIRIVQIEAKPNTLVLP  
30 KHADADNILVIQQGQATVTVANGNNRKSFNLDENGHALRIPSGFISYI

LNRHDNQNLRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGFSRNT  
 LEAAFNAEFNEIRRVLLEENAGGEQEERGQRRWSTRSSENNEGIVV  
 KVSKEHVEELTKHAKSVSKKGSEEEGDITNPINLREGEPDLSNNFGK  
 LFEVKPDKKNPQLQDLDMLTCVEIKEGALMLPHFNSKAMVIVVV  
 5 NKGTGNLELVAVRKEQQQRGRREEEDEDEEEEESNREVRRYTAR  
 LKEGDVFIMPAAHPVAINASSELHLLGFGINAENNHRIFLAGDKDN  
 VIDQIEKQAKDLAFPGSGEQVEKLIKQNQKESHFVSARPQSQSQPSSP  
 EKESPEKEDQEEENQGGKGPLLSILKAFN

10 **Ragweed**

Ambrosia sequences

113478 Amb a 1  
 MGIKHCCYILYFTLALVTLLQPVRSAEDLQQILPSANETRSLTTCGT  
 15 YNIIDGCWRGKADWAENRKALADCAQGFAKGTIGGKDGDITYTVTS  
 ELDDD VAN PKEGTLRGAAQNRPLWIFARDMViRLDRELAINNDK  
 TIDGRGAKVEIINAGFAIYNVKNIIHNIMHDIVVNPGLIKSHDGPP  
 VPRKGSDGDAIGISGGSQIWIDHCSLSKAVDGLIDAKHGSTHFTVSN  
 CLFTQHQYLLLFWDFDERGMLCTVAFNKFTDNVDQRMPNLRHGF  
 20 VQVVNNNYERWGSYALGGSAGPTILSQGNRFLASDIKKEVVGRYG  
 ESAMESINWNWRSYMDVFENGAIIFVPSGVDPVLTPEQNAGMIPAE  
 PGEAVRLLTSSAGVLSCQPGAPC

113479 Amb a 2  
 25 MGIKHCCYILYFTLALVTLVQAGRGEVDILPSPNDTRSLQGCE  
 AHNIIDKCWRCKPDWAENRQALGNCAQGFGKATHGGKWGDIYM  
 VTSDQDDDVNPKEGTLRGATQDRPLWIFQRDMIIYLQQEMVVT  
 SDKTIDGRGAKVELVYGGITLMNVKNVIIHNIDIHDVRVLPGGRIKS  
 NGGPAIPRHQSDGDAIHVTGSSDIWIDHCTL SKSF DGLVDVNWGST  
 30 GVTISNCKFTHHEKA VLL GASD THF QDL KMHV TLAY NIFT NTVHE

RMPRCRGFFQIVNNFYDRWDKYAIGGSSNPTILSQGNKFVAPDFIY  
KKNVCLRTGAQEPEWMTWNWRTQNDVLENGAIFVASGSDPVLT  
EQNAGMMQAEPGDMVPQLTMNAGVLTCSGPAPC

5 113477 Amb a 1.3

MGIKQCCYILYFTLALVALLQPVRSAEGVGEILPSVNETRSLQACEA  
LNIIDKCWRGKADWENNQRQALADCAQGFAKGTYGGKWGDVYTV  
TSNLDDD VANPKEGTLRFAAAQRPLWIIFKNDMVINLNQELVV  
SDKTIDGRGVKVEIINGGLTLMNVKNIIHNINIHDVKVLPGGMIKSN  
10 DGPPILRQASDGDTINVAGSSQIWIDHCSLSKSF DGLVDVTLGSTHV  
TISNCKFTQQSKAILLGADDTHVQDKGMLATVAFNMFTDNVDQR  
MPRCRGFFQVVNNNYDRWGT AIGGSSAP TILCQGNRFLAPDDQI  
KKNVLARTGTGAAESMAWNWRSDKLLENGAIFVTSGSDPVLT  
QSAGMIPAEPGEAAIKLTSSAGVFSCHPGAPC

15

113476 Amb a 1.2

MGIKHCCYILYFTLALVTLLQPVRSAEDVEEFLPSANETRRSLKACE  
AHNIIDKCWRCKADWANNRQALADCAQGFAKGTYGGKHGDVYT  
VTSKD DDVANPKEGTLRFAAAQRPLWIIFKRN MVIHLNQELVV  
20 NSDKTIDGRGVVNIVNAGLTL MNVKNIIHNINIHDIKVC PGGMIKS  
NDGPPILRQQSDGDAINVAGSSQIWIDHCSLSKASDGLLDITLGSSH  
TVSNCKFTQHFVLLGADDTHYQDKGMLATVAFNMFTDHVDQR  
MPRCRGFFQVVNNNYDRWGT AIGGSSAP TILSQGNRFFAPDDIIK  
KNVLARTGTGNAESMSWNWRTDRDLLENGAIFLPSGSDPVLT  
PEQ  
25 KAGMIPAEPGEAVRLTSSAGVLSCHQGAPC

113475 Amb a 1.1

MGIKHCCYILYFTLALVTLLQPVRSAEDLQEILPVNETRRLTTSGAY  
NIIDGCWRGKADWAENRKALADCAQGFGKGTVGGKDGDIYTVTS  
30 ELDDD VANPKEGTLRGAAQRPLWIIFERDMVIRLDKEMVVNSD

KTIDGRGAKVEIINAGFTLNGVKNVIIHNINMHDVKVNPGGLIKSND  
 GPAAPRAGSDGDAISISGSSQIWIDHCSLSKSVDGLVDAKLGTRLT  
 VSNSLFTQHQFVLLFGAGDENIEDRGMLATVAFTNTFDNVDQRMP  
 RCRHGFFQVVNNYDKWGSYAIGGSASPTILSQGNRFCAPDERSKK  
 5 NVLGRHGEAAAESMKWNWRTNKDVLENGAIFVASGVDPVLTPEQ  
 SAGMIPAEPGESALSLTSSAGVLSCQPGAPC

### Cedar sequences

10 493634 Cry j IB precursor

MDSPLCLVALLVFSFVIGSCFSNDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGKDLYTVTNSDDDPVNPPGTLRYGATRDRPLWI  
 IFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVSNV  
 IIHGLYLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNIWI  
 15 DHNSFSNSSDGLVDVTLTSTGVTISNNLFFNHHKVMMSLGHDDAYSD  
 DKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNYDPWTIYAI  
 GGSSNPTILSEGSFTAPNESYKKQVTIRIGCKTSSCSNWVWQSTQ  
 DVFYNGAYFVSSGKYEGGANIYTKEAFNVENGATPHLTQNAGVL  
 TCSLSKRC

20

493632 Cry j IA precursor

MDSPLCLVALLVLSFVIGSCFSNDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGKDLYTVTNSDDDPVNPAAPGTLRYGATRDRPL  
 WIIFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVS  
 25 NVIIHGLHLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNI  
 WIDHNSFSNSSDGLVDVTLSSTGVTISNNLFFNHHKVMLLGHDDAY  
 SDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNYDPWTIY  
 AIGGSSNPTILSEGSFTAPNESYKKQVTIRIGCKTSSCSNWVWQST  
 QDVFYNGAYFVSSGKYEGGANIYTKEAFNVENGATPQLTKNAGV  
 30 LTCSSLKRC

00050000-0000-0000-0000-000000000000

1076242 Cry j II precursor - Japanese cedar  
MAMKLIAPMAFLAMQLIIMAAAEDQSAQIMLDVVEKYLRSNRL  
RKVEHSRHDAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKNPS  
5 AMLLVPGSKKFVVNNLFFNGPCQPHFTFKVDGIIAAYQNPASWKN  
NRIWLQFAKLTGFTLMKGVIDGQGKQWWAGQCKWVNGREICND  
RDRPTAIKFDFSTGLIIQGLKLMNSPEFHLVFGNCEGVKIIGISITAPR  
DSPNTDGIDIFASKNFHLQKNTIGTGDCAIGTGSSNIVIEDLICGP  
GHGISIGSLGRENSRAEVSYVHVNNGAKFIDTQNGLRIKTWQGGSGM  
10 ASHIIYENVEMINSEN PILINQFYCTSASACQNQRSAVQIQDVTYKNI  
RGTSATAAAIQLKCSDSMPCKDIKLSDISLKLTSGKIASCLNDNANG  
YFSGHVIPACKNLSPSAKRKE SKSHKHPKTVMVENMRAYDKGNRT  
RILLGSRPPNCTNKCHGCSPCKAKLVIVHRIMPQEYYPQRWIC SCHG  
KIYHP  
15  
1076241 Cry j II protein - Japanese cedar  
MAMKFIAPMAFVAMQLIIMAAAEDQSAQIMLDSDIEQYLRSNRL  
KVEHSRHDAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKKPSA  
MLLVPGNKKFVVNNLFFNGPCQPHFTFKVDGIIAAYQNPASWKNN  
20 RIWLQFAKLTGFTLMKGVIDGQGKQWWAGQCKWVNGREICNDR  
DRPTAIKFDFSTGLIIQGLKLMNSPEFHLVFGNCEGVKIIGISITAPRD  
SPNTDGIDIFASKNFHLQKNTIGTGDCAIGTGSSNIVIEDLICPG  
HGIGISIGSLGRENSRAEVSYVHVNNGAKFIDTQNGLRIKTWQGGSGMA  
SHIIYENVEMINSEN PILINQFYCTSASACQNQRSAVQIQDVTYKNIR  
25 GTSATAAAIQLKCSDSMPCKDIKLSDISLKLTSGKIASCLNDNANGY  
FSGHVIPACKNLSPSAKRKE SKSHKHPKTVMVKNMGA YDKGNRTRI  
LLGSRPPNCTNKCHGCSPCKAKLVIVHRIMPQEYYPQRWMCSRHG  
KIYHP  
30 541803 Cry j I precursor - Japanese cedar

MDSPCLVALLVLSFVIGSCFSNDNPIDSCWRGDSNWAQNRMKLADC  
AVGFGSSTMGGKGGDLYTVTNSDDDPVNPPGTLRYGATRDRPLWI  
IFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVSNV  
IIHGLHL YGCSTSVLGNVLINESFGVEPVHPQDGDA~~T~~LRTATNIWI  
5 DHNSFSNSSDGLVDVTLSTGVTISNNLFFNHHKVMLLGHDAYSD  
DKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNYDPWTIYAI  
GGSSNPTILSEGNSFTAPNESYKKQVTIRIGCKTSSCSNWVWQSTQ  
DVFYNGAYFVSSGKYEGGNIYT~~K~~KEAFNVENGATPQLTKNAGVL  
TCSLSKRC

10

541802 Cry j I precursor- Japanese cedar

MDSPCLVALLVFSFVIGSCFSNDNPIDSCWRGDSNWAQNRMKLADC  
AVGFGSSTMGGKGGDLYTVTNSDDDPVNPA~~G~~PGTLRYGATRDRPL  
WIIFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRV  
15 NVIIHGLLYGCSTSVLGNVLINESFGVEPVHPQDGDA~~T~~LRTATNI  
WIDHNSFSNSSDGLVDVLTSTGVTISNNLFFNHHKVMSLGHDAY  
SDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNYDPWTIY  
AIGGSSNPTILSEGNSFTAPNESYKKQVTIRIGCKTSSCSNWVWQST  
QDVFYNGAYFVSSGKYEGGNIYT~~K~~KEAFNVENGATPHLTQNAGV  
20 LTC~~S~~LSKRC

Dog

Canis sequences:

25 Can f 1

MKTLLTIGFSLIAILQAQDTPALGKDTVAVSGKWYLKAMTADQE  
VPEKPDSVTPMILKAQKGGNLEAKITMLTNGQCQNITVVLHKTSEP  
GKYTA~~Y~~E~~G~~Q~~R~~VVF~~I~~Q~~P~~S~~P~~VRDH~~Y~~ILYCEGELHGRQIRMAKLLGRDP  
EQSQEALDFREFSRAKGLNQEILELAQSETCSPGGQ

30

Serum albumin fragment

EAYKSEIAHRYNDLGEEHFRGLVL

Serum albumin fragment

5 LSSAKERFKCASLQKFGDRAFKAWSVARLSQRFPKADFAEISKVVT  
DLTKVHKECCHGDLLECADDRADLAKYMCENQDSISTKLKECCDK  
PVLEKSQCLAEVERDELPGDLP SLAADFVEDKEVCKNYQEAKDVF  
LGTFLYEYSRRHPEYSVSLLLRAKEYEATLEKCCATDDPPTCYAK  
VLDEFKPLVDEPQNLVKTNCELFEKLGEYGFQNALLVRYTKKAPQ  
10 VSTPTLVVEVSRKLGKVGTKCCKKPESERMSCADDFLS

Can f 2

15 MQLLLLTVGLALICGLQAQEGNHEEPQGGLEELSGRWH SVALASN  
KSDLIKPWGHFRVFIHSMSAKDGNLHGDILIPQDGQCEKVSLTAFKT  
ATSNKFDLEYWGHNDLYLAEVDPKSYLILYMINQYNDDTSLVAHL  
MVRDLSRQQDFLPAFESVCEDIGLHKDQIVVLSDDDRCQGSRD

Additional dog allergen protein (NCBI entrez accession):

20 1731859

**Horse**

Equus sequences:

25 1575778 Equ c1

MKLLLLCLGLILVCAQQEENSDVAIRNFDISKISGEWYSIFLASDVK  
EKIEENGSMRVFVDVIRALDNSSLYAEYQTKVNGECTEFPMVFDKT  
EEDGVYSLNYDGYNVRISEFENDEHIILYLVNFDRPFQLFEFYA  
REPDVSPEIKEEVKIVQKRGIVKENIIDLT KIDRCFQLRGNGVAQA

3121755 Equ c 2

SQXPQSETDYSQLSGEWNTIYGAASNIXK

5 Euroglyphus (mite)

Euroglyphus sequences:

Eur m 1 (variant)

TYACSINSVSLPSELDLRLRTVTPIRMQGGCGSCWAFSGVASTESA  
10 YLAYRNMSLDLAEQELVDCASQNGCHGDTIPRGIEYIQQNGVVQE  
HYYPYVAREQSCHRPNQAQRYGLKNYCQISPPDSNKIRQALTQTHTA  
VAVIIGIKDLNAFRHYDGRTIMQHDNGYQPNYHAVNIVGYGNTQG  
VDYWIVRNSWDTTWGDNGYGYFAANINL

15 Eur m 1 (variant)

TYACSINSVSLPSELDLRLRTVTPIRMQGGCGSCWAFSGVASTESA  
YLAYRNMSLDLAEQELVDCASQNGCHGDTIPRGIEYIQQNGVVQE  
HYYPYVAREQSCHRPNQAQRYGLKNYCQISPPDSNKIRQALTQTHTA  
VAVIIGIKDLNAFRHYDGRTIMQHDNGYQPNYHAVNIVGYGNTQG  
20 VDYWIVRNSWDTTWGDNGYGYFAANINL

Eur m 1 (variant)

ETNACCSINGNAPAEIDLQMRTVTPIRMQGGCGSCWAFSGVAATES  
AYLA YRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQQHNGVVQE  
25 SYYRYVAREQSCRRPNAQRFGISNYCQIYPPNANKIREALAQTHSAI  
AVIIGIKDLDAFRHYDGRTIIQRDNGYQPNYHAVNIVGYSNAQGVD  
YWIVRNSWDTNWGDNGYGYFAANIDL

Eur m 1 (variant)

30 ETSACRINSVNPSELDLRLRTVTPIRMQGGCGSCWAFSGVAATES

AYLAYERNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQQNGVVEE  
RSYPYVAREQQCRRPNSQHYGISNYCQIYPPDVKQIREALTQTHTAI  
AVIIGIKDLRAFQHYDGRTIIQHDNGYQPNEYHAVNIVGYGSTQGVD  
YWIVRNSWDTTWGDSGYGYFQAGNNL

5

Poa (grass) sequences

113562 POLLEN ALLERGEN POA P 9

MAVQKYTVALFLVALVVGPAASYAADLSYGAPATPAAPAAGYTP  
10 AAPAGAAPKATTDEQKMIEKINVGFKA AVAAAGGVPAANKYKTFV  
ATFGAASNKAFAEALSTEPKGAAVDSSKAALTSKLDAAYKLAYKS  
AEGATPEAKYDDYVATLSEALRIIAGTLEVHGVKPAAEEVKATPAG  
ELQVIDKVDAAFKVAATAANAAPANDKFTVFEAAFNDAIKASTGG  
AYQSYKFIPALEAAVKQSYAATVATAPAVKYTVFETALKKAITAMS  
15 QAQKAAKPAAAATGTATAAVGAATGAATAAAGGYKV

113561 POA P 9

MAVHQYTVALFLAVALVAGPAASYAADVGYGAPATLATPATPAA  
PAAGYTPAAPAGAAPKATTDEQKLIEKINAGFKAAVAAAAGVPAV  
20 DKYKTFVATFGTASNKAFAEALSTEPKGAAAASSNAVLTSKLDAAYK  
LAYKSAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAGEEV  
VKAIPAGELOQVIDKVDAAFKVAATAANAAPANDKFTVFEAAFNDA  
IKASTGGAYQSYKFIPALEAAVKQSYAATVATAPAVKYTVFETALK  
KAITAMSQAQKAAKPAAAATATGAVGAATGAVGAAATGAATAA  
25 AGGYKTGAATPTAGGYKV

113560 POA P 9

MDKANGAYKTALKAAASAVAPAEKFPVFQATFDKNLKEGLSGPDA  
VGFAKKLDIFIQTSYLSTKAAEPKEKFDFVLSLTEVLRFMAGAVK  
30 APPASKFPAKPAPKVAAYTPAAPAGAAPKATTDEQKLIEKINVGF

AAVAAAAGVPAASKYKTFVATFGAASNKAFAEALSTEPKGAAVAS  
SKAVLTSKLDAAVKLAYKSAEGATPEAKYDAYVATLSEALRIIAGT  
LEVHGVKPAAEEVKAIPAGELQVIDKVDAAFKVAATAANAAPAND  
KFTVFEAAFNDAIKASTGGAYQSYKFIPALEAAVKQSYAATVATAP  
5 AVKYTVFETALKKAITAMSQAQKAQPKAAAVTGTATSAVGAATGA  
ATAAAGGYKV

### Cockroach sequences

10 2833325 Cr p1

MKTALVFAAVVAFVAARFPDHKDYKQLADKQFLAKQRDVRLFH  
RVHQHNILNDQVEVGIPMTSKQTSAVVPPSGEAVHGLQEGHARP  
RGEPFSVNYEKREQAIMLYDLLYFANDYDTFYKTACWARDRVN  
EGMFMFMSFSIAVFHRDDMQGVMMLPPPDEVYPYLFDHDVIHMAQ  
15 KYWMKNAGSGEHHSHVIPVNFTLRTQDHLLAYFTSDVNLNAFNTY  
YRYYYPSWYNTTLYGHNIIDRRGEQFYTYKQIYARYFLERLSNDLP  
DVYPFYYSKPVKSAYNPNLRYHNGEEMPVRPSNMVVTNFDLYIA  
DIKNEYKRVEDAIDFGYAFDEHMKPHSLYHDVHGMEYLAADMIEG  
NMDSPNFYFYGSIYHMYHSMIGHIVDPYHKMGLAPSLEHPETVLR  
20 DPVFYQLWKRVDHDFQKYKNRLPRYTHDELAFEGVKVENVDVGK  
LYTYFEQYDMSLDMAVYVNNVDQISNVQLAVRLNHKPFTYNIE  
VSSDKAQDVYVAVFLGPKYDYLGREYDLNDRRHYFVEMDRFPYH  
VGAGKTVIERNSHDSNIIAPERDSYRTFYKKVQEAYEGKSQYYVDK  
GHNCGYPENLLIPKGKKGGQAYTFYVIVTPYVKQDEHDFEPYNY  
25 KAFSYCGVGSERKYPDNKPLGYPFDRKIYSNDFYTPNMYFKDVIIIF  
HKKYDEVGVQGH

2231297 Cr p2

INEIHSIIGLPPFVPPSRRHARRGVGINGLIDDVIAILPVDELKALFQE  
30 KLETSPDFKALYDAIRSPEFQSIISTLNAMQRSEHHQNLRDKGVDVD

HFIQLIRALFGLSRAARNLQDDLNDLHSLEPISPRHRHGLPRQR  
SARVSAYLHADDFHKIITTIEALPEFANFYNFLKEHGLDVVDYINEI  
HSIIGLPPFVPPSRRHARRGVGINGLIDDVIAILPVDELKALFQEKL  
SPDFKALYDAIRSPEFQSIISTLNAMPEYQELLQNLRDKGVDVDHFI  
5 RVDQGTLRTLSSGQRNLQDDLNDLALIPTDQILAIAMDYLANDAE  
VQELVAYLQSDDFHKIITTIEALPEFANFYNFLKEHGLDVVDYINEI  
HSIIGLPPFVPPSQRHARRGVGINGLIDDVIAILPVDELKALFQEKL  
SPDFKALYDAIDLRSSRA

10 1703445 Bla g 2

MIGLKLVTVLFAVATITHAAELQRVPLYKLVHVFIN  
TQYAGITKIGN  
QNFLTVDSTSCNVVVASQECVGGACVCPNLQKYEKL  
PKYISDG  
NVQVKFFDTGSAVGRGIEDSLTISNL  
TTSQQDIVLADELSQEVCILSA  
DVVVGIAAPGCPNALKGKTVLEN  
FVEENLIAPVFSIHARFQDGEH  
15 FGEIIFGGSDWKYVDGEFTYVPLVGDDSWKFRLDG  
VKGIDTTVAPA  
GTQAIIDTSKAIIVGPKA  
YVNPINEAIGCV  
VEKTT  
RICKLDCSKIPS  
LPDVT  
FVINGRN  
NFISSQYYIQ  
QNGNLC  
YSGFQPCGH  
SDHFF  
FIGDFF  
VDHYYSEFN  
WENKT  
MGFRSVE

SV

20

1705483 Bla g 4

AVLALCATDT  
LANEDCFRHE  
SLVPNLDYER  
FRGSWII  
AAGTSEALT  
QYKCWIDRF  
SYDDALVSKY  
TD  
TDSQGKNRT  
TIRGRTK  
FEGNKFT  
IDYN  
DKGKA  
FSAPYSV  
LATDY  
ENYAIVE  
GCPAA  
ANGHVIY  
VQIRFS  
VRRF  
25 HPKLGD  
KEMIQHY  
TLDQVNQ  
HKKAIE  
EDLKHF  
NLKYEDL  
HSTCH

2326190 Bla g 5

YKLTYCPV  
KALGE  
PIRFLL  
SYGE  
KDF  
E  
DYRF  
QE  
GDWP  
NLKP  
SMPFG  
KTPV  
LEIDG  
KQTH  
QSV  
AISRY  
LGKQ  
FGLSG  
KDD  
WEN  
LEID  
MIV  
DTIS

30 DFRAAIANYHY  
DADENS  
KQKKWDPL  
KKETIP  
YYTKKF  
DEVVKANG

GYLAAGKLTWADFYVAILDYLNHMAKEDLVANQPNLKALREKV  
LGLPAIKAWVAKRPPTDL

Additional cockroach sequences (NCBI Entrez accession numbers):

5 2580504; 1580797; 1580794; 1362590; 544619; 544618; 1531589;  
1580792; 1166573; 1176397; 2897849.

Allergen (general) sequences:

NCBI accession numbers

10

2739154; 3719257; 3703107; 3687326; 3643813; 3087805; 1864024;  
1493836; 1480457; 2598976; 2598974; 1575778; 763532; 746485;

163827; 163823; 3080761; 163825; 3608493; 3581965; 2253610;  
2231297 ; 2897849; 3409499; 3409498; 3409497; 3409496; 3409495;

15 3409494; 3409493; 3409492; 3409491; 3409490; 3409489; 3409488;  
3409487; 3409486; 3409485; 3409484; 3409483; 3409482; 3409481;  
3409480; 3409479; 3409478; 3409477; 3409476; 3409475; 3409474;  
3409473; 3409472; 3409471; 3409470; 3409469; 3409468; 3409467;  
3409466; 3409465; 3409464; 3409463; 3409462; 3409461; 3409460;

20 3409459; 3409458; 3409457; 3409456; 3318885; 3396070 ; 3367732;  
1916805; 3337403; 2851457; 2851456; 1351295; 549187; 136467;  
1173367; 2499810; 2498582; 2498581; 1346478; 1171009; 126608;  
114091; 2506771; 1706660; 1169665; 1169531; 232086; 416898;  
114922; 2497701; 1703232; 1703233; 1703233; 1703232; 3287877;

25 3122132; 3182907; 3121758; 3121756; 3121755; 3121746; 3121745;  
3319925; 3319923; 3319921; 3319651; 3318789; 3318779; 3309647;  
3309047; 3309045; 3309043; 3309041; 3309039; 3288200; 3288068;  
2924494; 3256212; 3256210; 3243234; 3210053; 3210052; 3210051;  
3210050; 3210049; 3210048; 3210047; 3210046; 3210045; 3210044;

30 3210043; 3210042; 3210041; 3210040; 3210039; 3210038; 3210037;

3210036; 3210035; 3210034; 3210033; 3210032; 3210031; 3210030;  
3210029; 3210028; 3210027; 3210026; 3210025; 3210024; 3210023;  
3210022; 3210021; 3210020; 3210019; 3210018; 3210017; 3210016;  
3210015; 3210014; 3210013; 3210012; 3210011; 3210010; 3210009;  
5 3210008; 3210007; 3210006; 3210005; 3210004; 3210003; 3210002;  
3210001; 3210000; 3209999; 3201547; 2781152; 2392605; 2392604;  
2781014; 1942360; 2554672; 2392209; 3114481; 3114480; 2981657;  
3183706; 3152922 ; 3135503 ; 3135501; 3135499; 3135497; 2414158;  
10 1321733; 1321731; 1321728; 1321726; 1321724; 1321722; 1321720;  
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; 386678 ; 169865.

Example 7: Desensitisation using multiple overlapping peptides  
25 (MOP) from Fel d I

We have obtained data with multiple overlapping peptides (MOP) which are derived from the sequence of Fel d I and include the three FC1P peptides. Originally, 16 peptides spanning both chain 1 and chain 2 of the  
30 Fel d I molecule were designed in order to increase the percentage of

individuals reacting to the peptide injection. By using peptides covering the entire molecule, we believed that we would cover more MHC-peptide pairings and thus get more reactors. Of the 16 peptides, the first three of chain 2 displayed poor solubility in aqueous solution and were excluded  
5 from the *in vivo* preparation termed MOP. The sequences of the MOP peptides and how they relate to the parent molecule are given in Figure 9.

We have carried-out a dose ranging study with this preparation to determine an appropriate dose to be used in a planned clinical trial in  
10 which four injections of increasing dose will be given over a two week period. For the dose ranging study, three doses have been tested: 1 $\mu$ g (of each of the 13 peptides in a mixture), 2.5 $\mu$ g and 5 $\mu$ g.

Four cat asthmatic individuals received the 1 $\mu$ g dose. One of them  
15 developed a LAR which was similar to those induced with FC1P. Five individuals received 2.5 $\mu$ g and again one developed a LAR. At 5 $\mu$ g, eight individuals were tested and four developed a LAR. This demonstrates the dose response effect that we expected and, more importantly, shows that the MOP preparation produces a similar effect to the FC1P preparation.  
20 An example of a LAR induced by MOP can be seen in Figure 10.

Rather than move to a higher dose which may give a higher percentage of LAR reactors, we have decided to use the 5 $\mu$ g dose as the starting dose for the trial. From the number of peptides in the MOP preparation and the  
25 observed dose response, it might be expected that some of the non-LAR reactors at 5 $\mu$ g might develop a LAR at a higher dose, ie have the appropriate MHC molecules to recognise the peptides but experienced a “sub-clinical” reaction. For this reason, we decided to investigate the cutaneous late phase reaction to whole allergen extract as an alternative  
30 clinical outcome. Basically, if whole allergen extract is injected

intradermally (in our case into the forearm) in an atopic allergic individual, an immediate wheal and flare reaction will result (classical IgE mediated early allergic reaction) in about 15 minutes. This reaction is then followed by a delayed in-time phase reaction in the skin. Like the 5 lung reaction, this peaks at 6-9 hours and believed to be driven at least in part by T cells.

Previously, immunotherapy studies using conventional whole allergen extract have demonstrated that the size of this late phase skin reaction 10 decreases after several months of treatment.

We have measured these skin reactions before any peptide injection (ie at baseline) and we have measured them again in six patients (to date) who have had either one or two injections only of MOP. All six have reduced 15 reactions as shown in figure 11. These results are statistically significant with a p value of 0.036.

A further interesting observation was that some of these individuals did not develop a lung reaction (LAR) to the MOP injection but clearly their T 20 cells were activated by one or more of the peptides giving them a measurable reduction in reactivity to skin challenge with whole allergen extract (the latter being perhaps even more significant since the whole dander extract contains multiple proteins (including Fel d I) to which the patient may be sensitised).

25

As mentioned above, some (three) of the MOP injected individuals who developed lung reactions have received a second injection. As found with FC1P, these individuals did not develop reactions (an example can be seen in figure 10). Importantly, these two individuals received the second 30 injection several weeks (in one case about 4 months) after the first one.

This suggests that hyporesponsiveness induced after the first injection could last four months or more.

We also have other longitudinal data regarding the length of duration of  
5 the hyporesponsiveness from some of the FC1P patients. In this case,  
three patients who had received FC1P more than one year ago and  
experienced LAR's were rechallenged with the same dose. All three  
reacted with almost exactly the same magnitude as the initial reaction  
(Figure 12a, b & c). Of these three, one (Figure 12a) had received a  
10 second injection of FC1P a few weeks after the first and had displayed no  
LAR. Thus, peptides can induce a LAR which is followed by  
hyporesponsiveness which seems to last for four months (possibly more)  
but less than one year.

15 Finally, we now have three FC1P patients who have had one injection  
followed by a LAR which on reinjection was not seen (ie  
hyporesponsiveness). We also have the same finding in two MOP  
patients. We have had the areas under these curves analysed statistically.  
We have compared a control day (either saline injection or injection or  
20 whole cat extract, the latter does not induce a lung reaction only a skin  
reaction at the dose used), with the lung measurements (FEV1) after the  
first FC1P or MOP injection and after the second FC1P/MOP injection.

We have compared the mean values from spirometry by area under the  
25 curve analysis:

1. Control day vs peptide day 1 (we expect to see a significant difference i.e. there has actually been a significant reaction)
2. Control day vs peptide day 2 (do not expect a significant reaction since lung responses are back to normal)
- 30 3. Peptide day 1 vs peptide day 2 (expect a significant difference).

The results (p values) are:

1. p=0.0205
2. p=0.0930
- 5 3. p=0.0119

A p value of less than or equal to 0.05 is considered statistically significant.

10 Thus, there is a significant response to the peptides following the first injection (1) which is significantly different to the second injection (3) as the FEVI values appear to return to baseline. The difference between the control day and the second injection is not statistically significant (2).

## **CLAIMS**

1. A method of desensitising a patient to a polypeptide allergen the method comprising administering to the patient a peptide derived from the allergen wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.
- 10 2. A method according to Claim 1 wherein the peptide is included in a composition containing a plurality of peptides derived from the said allergen.
- 15 3. A method according to Claim 2 wherein the plurality of peptides derived from said allergen includes peptides for which restriction to Class II DR molecules DR2, DR3, DR4 and DR7 can be demonstrated, provided that such peptides can be derived from the allergen.
- 20 4. A method according to any one of Claims 1 to 3 wherein the patient possesses any one of the MHC Class II DR molecules DR2, DR3, DR4 or DR7.
- 25 5. A method according to any one of Claims 1 to 4 wherein the patient possesses the MHC Class II molecule DR4.
6. A method according to Claim 2 wherein the composition contains the Fel d I - derived peptides as given in SEQ ID Nos. 1, 2 and 3.
- 30 7. A method according to Claim 2 wherein the composition contains the soluble MHC Class II-restricted peptides of the Fel d I - derived

peptides described in Figure 9.

8. A composition comprising a plurality of peptides derived from a polypeptide allergen wherein for at least one of the peptides in the  
5 composition restriction to a MHC Class II molecule can be demonstrated and the composition is able to induce a late phase response in an individual possessing the given MHC Class II molecule.

9. A composition according to Claim 8 wherein at least one peptide is  
10 present in the composition for which restriction to each of MHC Class II DR molecules DR2, DR3, DR4 and DR7 can be demonstrated, provided that such peptides can be derived from the allergen.

10. A composition according to any one of Claims 8 or 9 for use in  
15 medicine.

11. A pharmaceutical formulation comprising a composition according to any one of Claims 8 or 9 and a pharmaceutically acceptable carrier.

20 12. A method according to Claim 1 wherein a composition according to any one of Claims 8 or 9 is administered to the patient.

13. Use of a peptide derived from a polypeptide allergen wherein restriction to a MHC Class II molecule possessed by a patient can be  
25 demonstrated by the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule in the manufacture of a medicament for desensitising a patient to said polypeptide allergen.

30 14. Use of a composition according to any one of Claims 8 or 9 in the

manufacture of a medicament for desensitising a patient to a polypeptide allergen.

15. A method according to any one of Claims 1 to 5 or 12, or a  
5 composition according to any one of Claims 8 or 9, or a use according to  
Claim 13 or 14, or a pharmaceutical preparation according to Claim 11  
wherein the polypeptide allergen is any one of Fel d I, Der p I, Der p II,  
Der fI or Der fII and allergens present in any of the following: grass, tree  
and weed (including ragweed) pollens; fungi and moulds; foods, stinging  
10 insects, the chironomidae (non-biting midges); spiders and mites, housefly,  
fruit fly, sheep blow fly, screw worm fly, grain weevil, silkworm,  
honeybee, non-biting midge larvae, bee moth larvae, mealworm,  
cockroach, larvae of *Tenibrio molitor* beetle, mammals such as cat, dog,  
horse, cow, pig, sheep, rabbit, rat, guinea pig, mice and gerbil.  
15

16. A composition according to any one of Claims 8 or 9, or a use  
according to Claim 13 or 14, or a pharmaceutical preparation according to  
Claim 11 wherein the polypeptide allergen is Fel d I and as given in SEQ  
ID Nos. 1, 2 and 3, or the composition contains the soluble MHC Class  
20 II-restricted peptides of the Fel d I - derived peptides described in Figure  
9.

17. A method of selecting a peptide for use as an immunotherapeutic  
agent for desensitising a patient to a polypeptide allergen capable of  
25 eliciting an allergic response in the patient, which patient possesses a  
particular MHC Class II molecule, the method comprising the steps of (1)  
selecting a candidate peptide derived from the polypeptide allergen, (2)  
determining whether the candidate peptide demonstrates restriction to the  
said MHC Class II molecule, and (3) determining whether the candidate  
30 peptide is able to induce a late phase response in an individual who

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possesses the said MHC Class II molecule.

18. A method according to Claim 17 wherein step (2) is carried out prior to step (3) and only candidate peptides which demonstrate restriction to the particular MHC Class II molecule are selected for testing in step (3).

5

19. A method according to Claim 18 or 19 wherein candidate peptides capable of inducing a late phase response and which demonstrate restriction to the particular MHC Class II molecule are selected as an immunotherapeutic agent.

10

20. A method according to any one of Claims 17 to 19 wherein determination of whether the candidate peptide demonstrates restriction to the said MHC Class II molecule is by using a T cell proliferation assay.

15

21. A method according to any one of Claims 17 to 20 wherein the allergen is selected from the group as defined in Claim 15.

20

22. A method according to any one of Claims 17 to 21 wherein in step (2) determination of whether the candidate peptide demonstrates restriction to the said MHC Class II molecule is by using the patient's cells in a T cell proliferation assay, and in step (3) determining whether the candidate peptide is able to induce a late phase response in the patient.

25

23. A method according to any one of Claims 17 to 22 wherein the MHC molecule is any one of HLA-DR, HLA-DP, HLA-DQ, or subclasses thereof.

30

24. A peptide when selected by any one of Claims 17 to 23.

25. A database of peptides characterised according to their ability to bind an MHC Class II molecule and induce a late phase response in an individual possessing the said MHC Class II molecule.

5

26. A peptide listed in a database according to Claim 25, for use in therapy.

27. A method for selecting a peptide for use as an immunotherapeutic  
agent for desensitising a patient to an allergen comprising the steps of:

15 a) tissue-typing the patient to determine MHC Class II type; and  
b) selecting, from a database of peptides which are known to bind to particular MHC Class II molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides capable of binding to the MHC Class II molecules possessed by the patient.

28. A method of determining an initial dose of an immunotherapeutic peptide for desensitising a patient to a polypeptide allergen, which peptide is derived from the allergen and wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC molecule, the method comprising (1) determining  
20 the dose which is able to generate an observable late phase response in a given proportion of individuals who possess the said MHC molecule and in whom the peptide is able to induce a late phase response and (2) selecting a lower dose which is incapable of inducing an observable late phase response in substantially all individuals who possess the said MHC  
25 molecule and in whom the peptide is able to induce a late phase response.

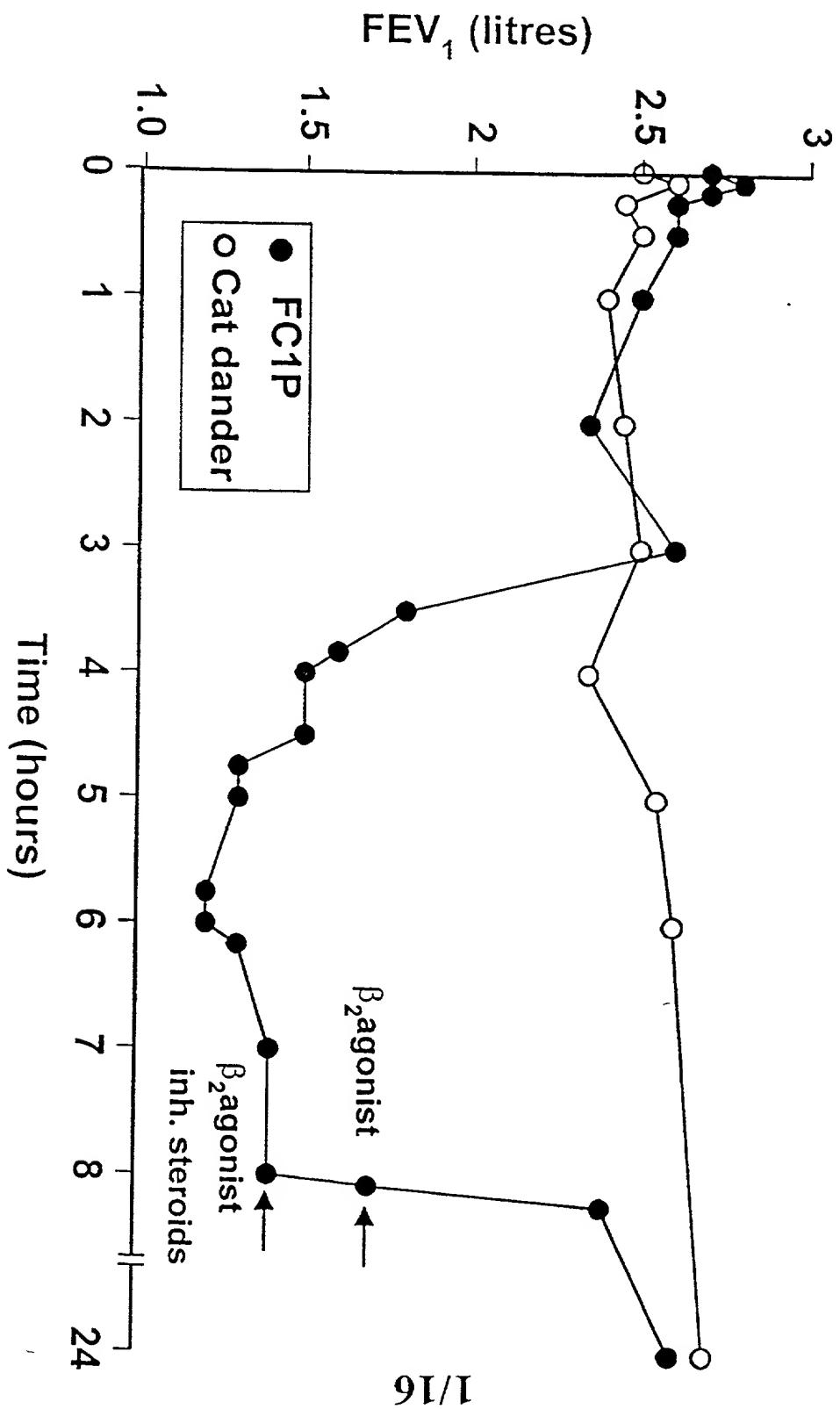
29. A method according to Claim 28 wherein in step (1) the given proportion is 50%.

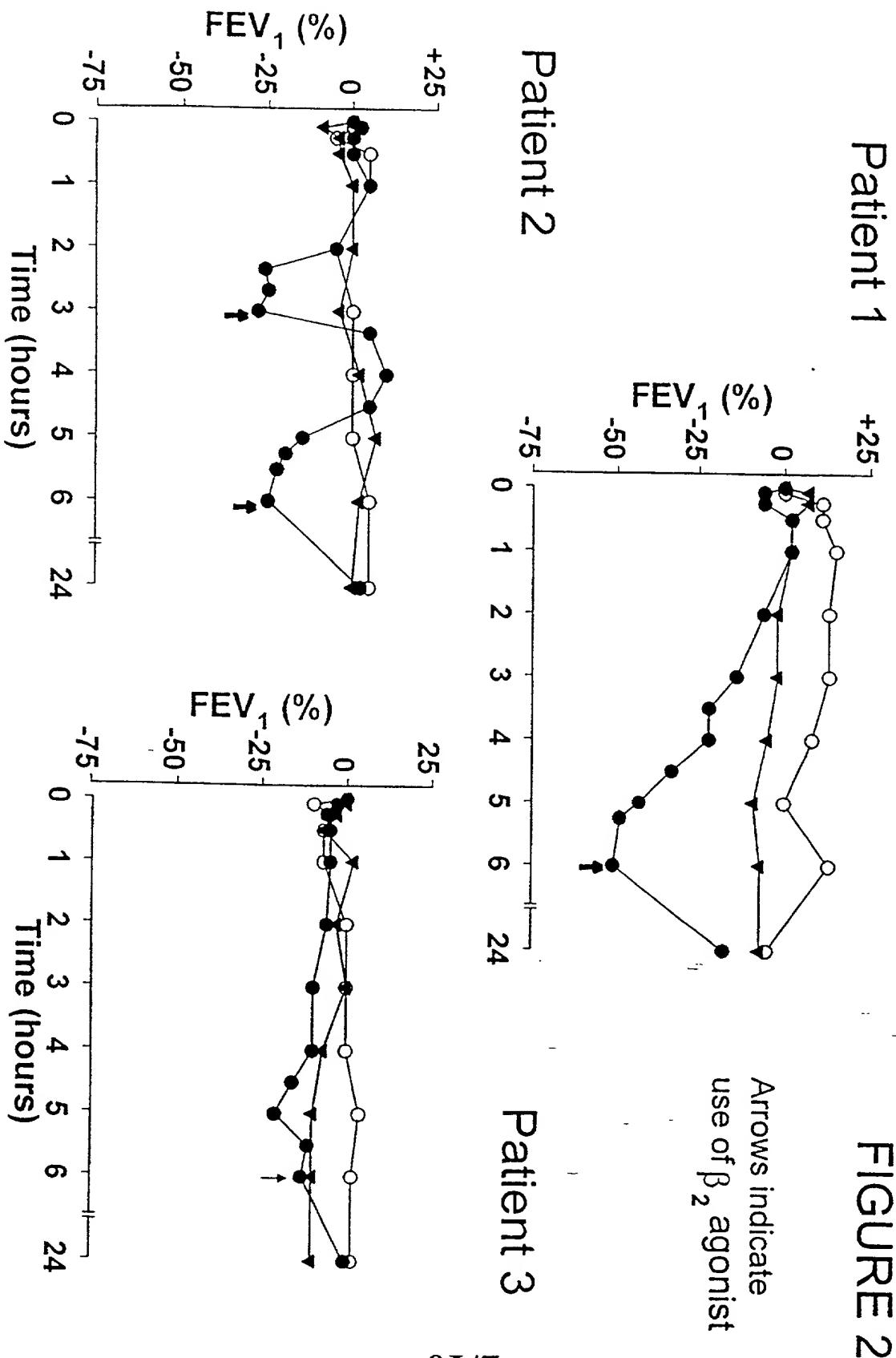
5 30. A method according to Claims 28 or 29 wherein the lower dose is 0.01% of the dose which is able to generate an observable late phase response in the given proportion of individuals.

10 31. A method according to any one of Claims 28 to 30 wherein in steps (1) and (2) the peptide is included in a plurality of peptides derived from the said allergen.

00000000000000000000000000000000

FIGURE 1



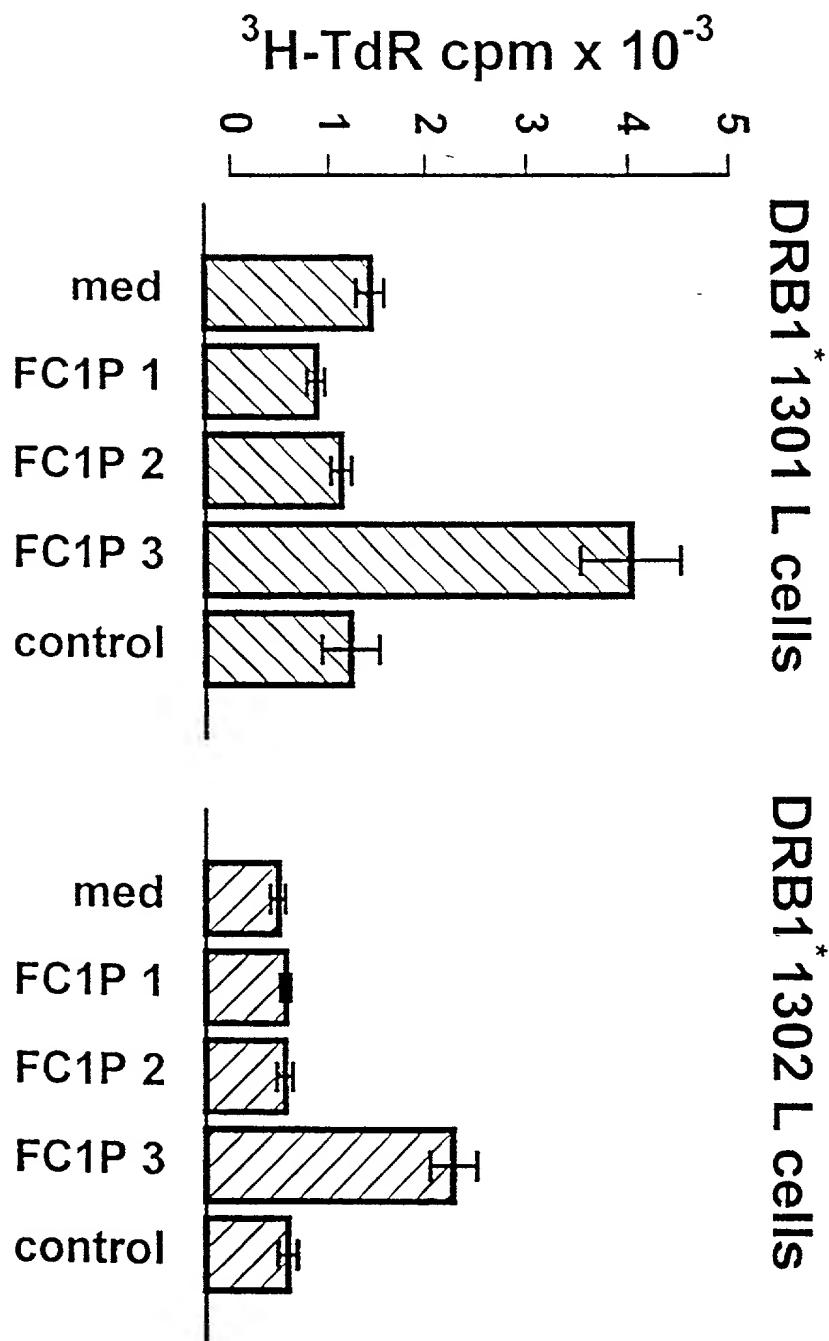


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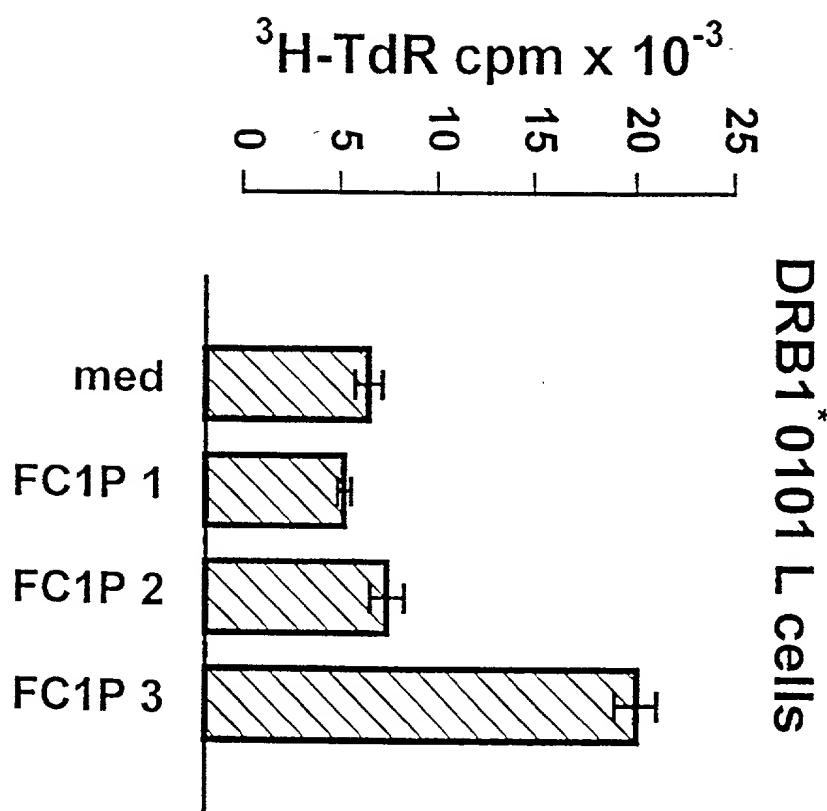
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FIGURE 3



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FIGURE 4

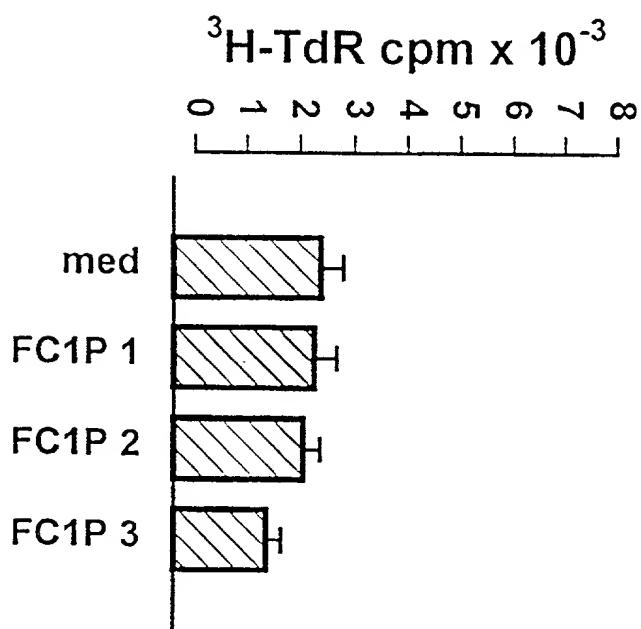


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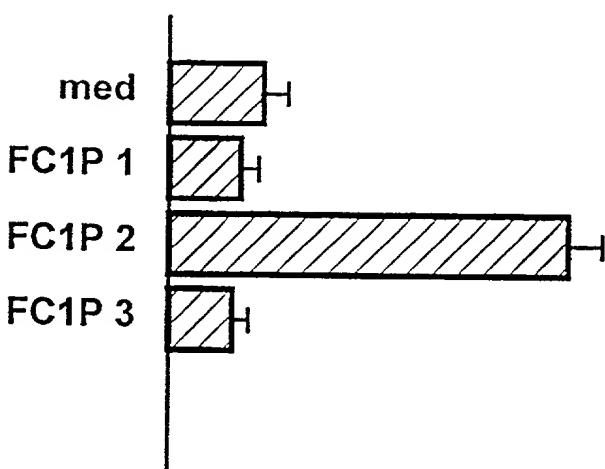
**FIGURE 5****DRB1\*0405/0408 Restriction of FC1P2 Response**

FC1P2 can be presented to 0408 T cells by 0405 but not 0404

a) DRB1\*0404 L cells



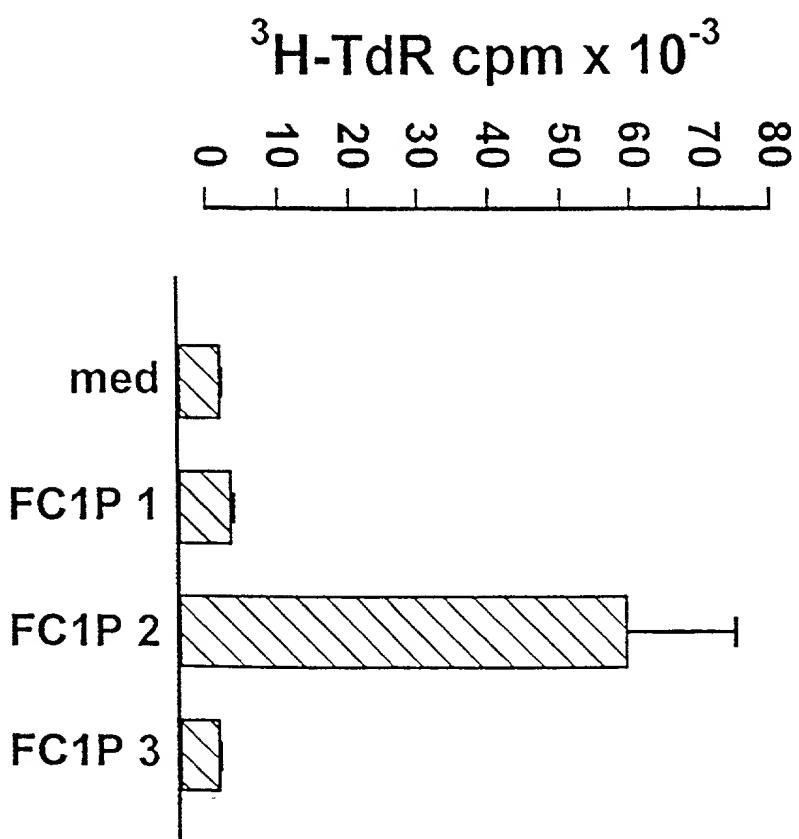
b) DRB1\*0405 L cells



Subject: DRB1\*0408

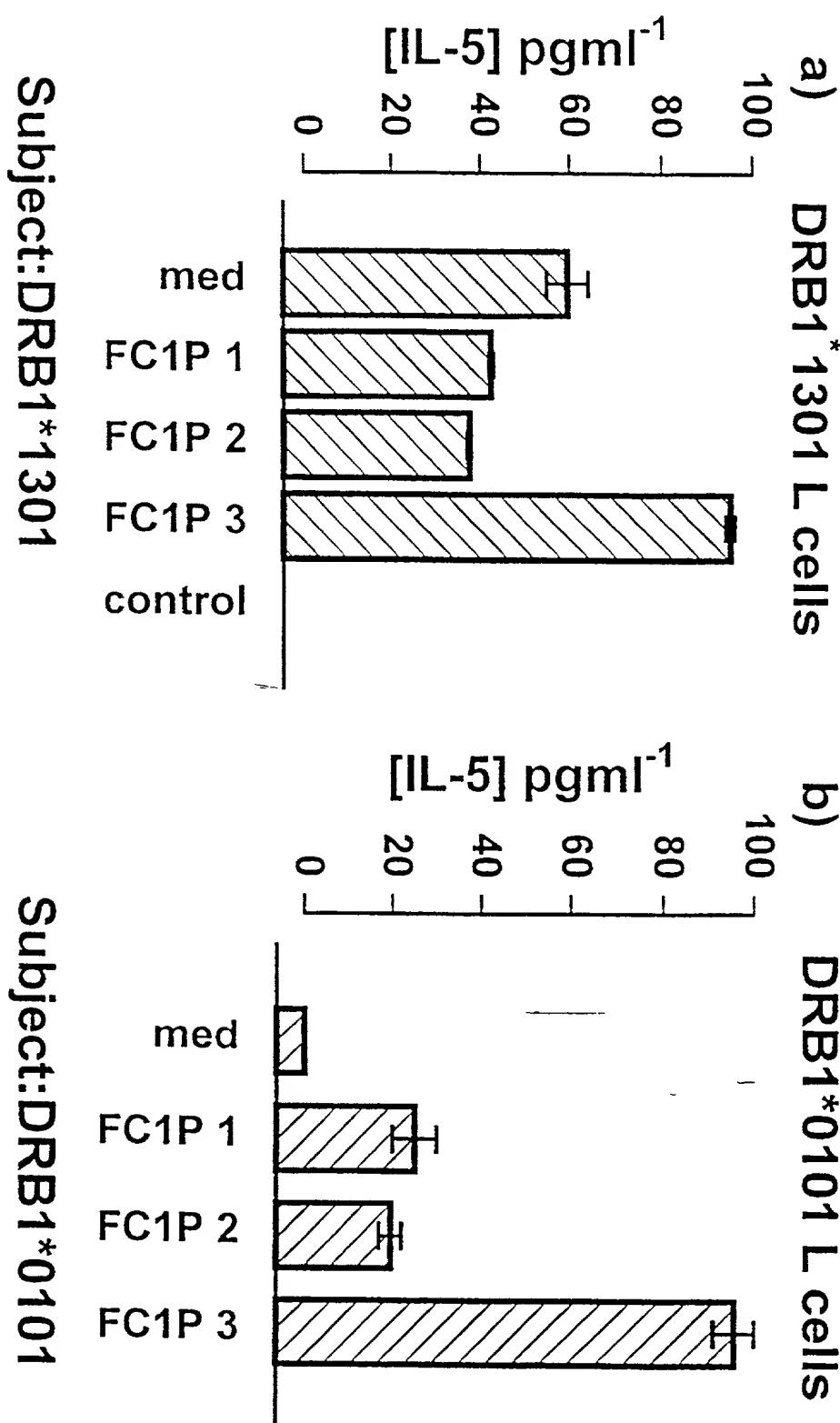
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FIGURE 6

**DRB1\*0405 L cells**

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FIGURE 7

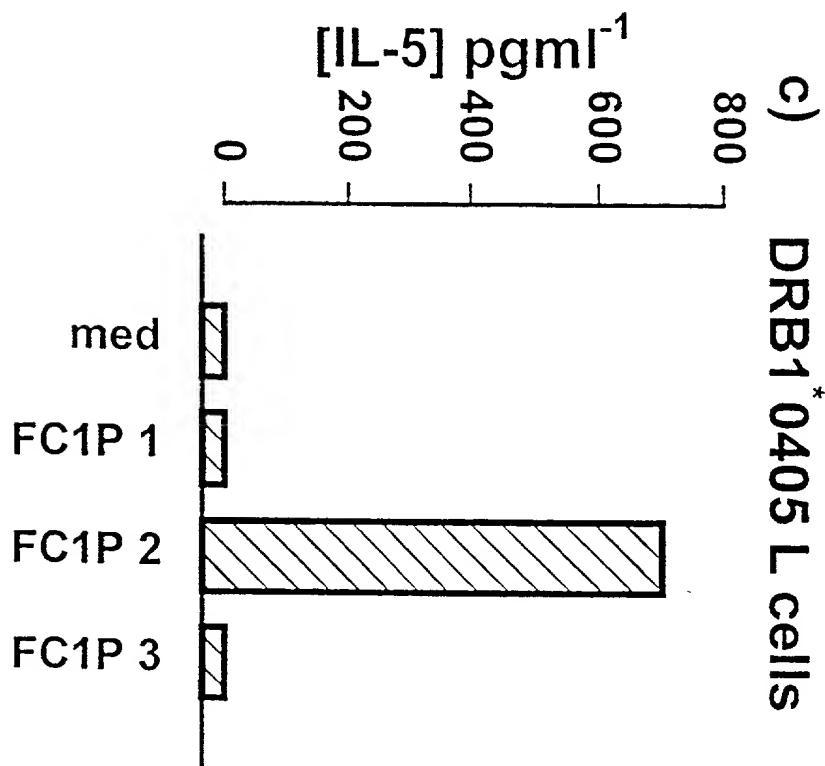


Subject:DRB1\*1301

Subject:DRB1\*0101

FIGURE 7

Subject:DRB1\*0405



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	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	A
1	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P						
2		R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S					
3			N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T				
4				D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W			
5					C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y		
6						Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
7							E	G	H	I	L	K	M	F	P	S	T	W	Y	V	A
8								G	H	I	L	K	M	F	P	S	T	W	Y	V	A
9									H	I	L	K	M	F	P	S	T	W	Y	V	A
10										I	L	K	M	F	P	S	T	W	Y	V	A
11											L	K	M	F	P	S	T	W	Y	V	A
12												K	M	F	P	S	T	W	Y	V	A
13													M	F	P	S	T	W	Y	V	A
14														F	P	S	T	W	Y	V	A
15															P	S	T	W	Y	V	A
16																S	T	W	Y	V	A
17																	T	W	Y	V	A
18																		W	Y	V	A
19																			Y	V	A
20																				V	A
21																					A
22																					
23																					
24																					
25																					
26																					
27																					
28																					

Figure 8 (Sheet 1 of 2). Hypothetical protein (top row) and peptides overlapping by one residue.

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	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	C	L	L	G
1																					
2																					
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8	R																				
9	R	N																			
10	R	N	D																		
11	R	N	D	C																	
12	R	N	D	C	Q																
13	R	N	D	C	Q	E															
14	R	N	D	C	Q	E	G														
15	R	N	D	C	Q	E	G	H													
16	R	N	D	C	Q	E	G	H	I												
17	R	N	D	C	Q	E	G	H	I	L											
18	R	N	D	C	Q	E	G	H	I	L	K										
19	R	N	D	C	Q	E	G	H	I	L	K	M									
20	R	N	D	C	Q	E	G	H	I	L	K	M	F								
21	R	N	D	C	Q	E	G	H	I	L	K	M	F	P							
22	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S						
23	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T						
24		D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W					
25			C	Q	E	G	H	I	L	K	M	F	P	S	T	W	C				
26				Q	E	G	H	I	L	K	M	F	P	S	T	W	C	L			
27					E	G	H	I	L	K	M	F	P	S	T	W	C	L	L		
28						G	H	I	L	K	M	F	P	S	T	W	C	L	L	G	

Figure 8 (Sheet 2 of 2). Hypothetical protein (top row) and peptides overlapping by one residue.

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**FIGURE 9    Multiple Overlapping Peptides (MOP)**

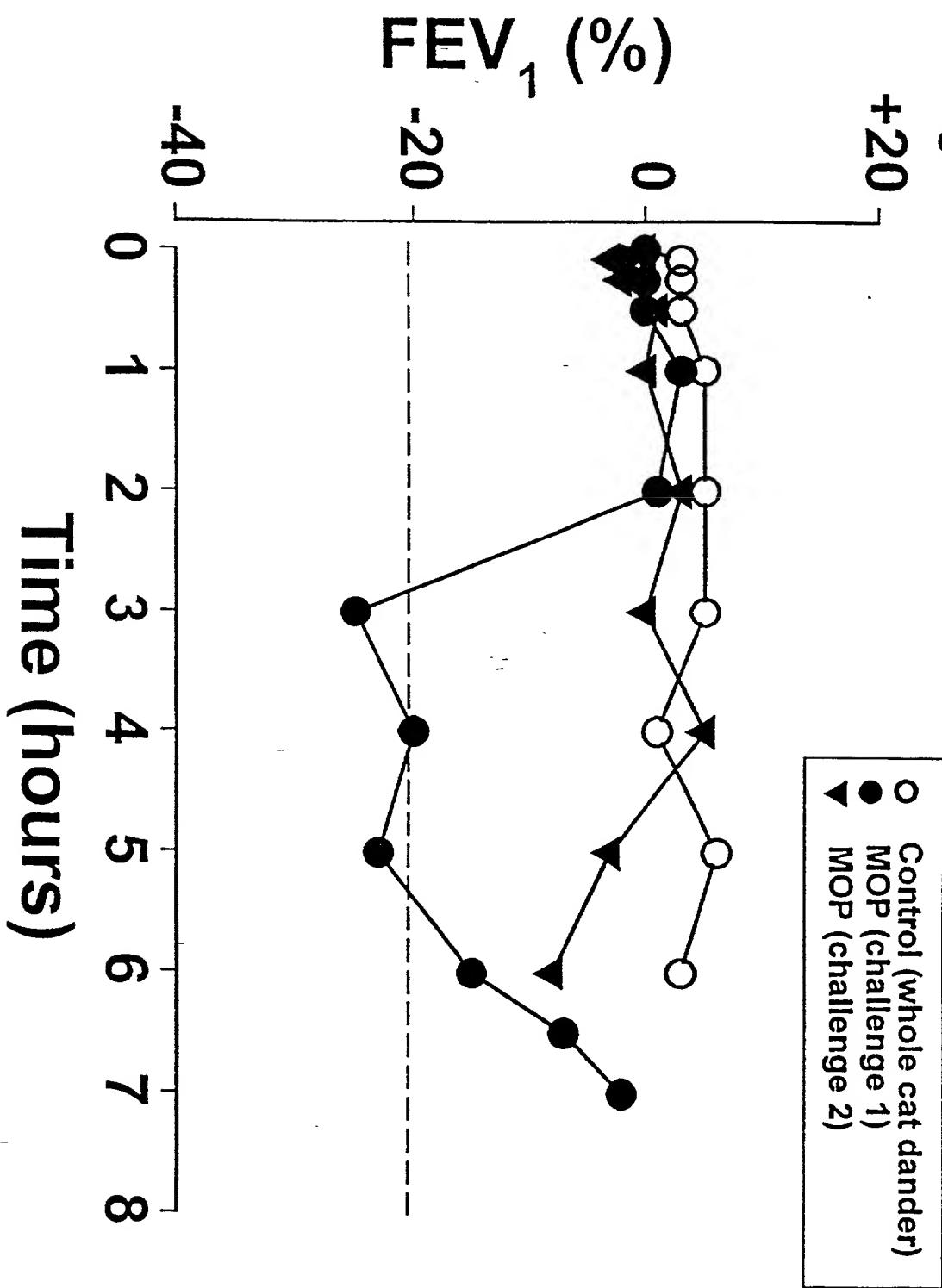
**Fel d I Chain 1**

EICPAVKRDVVLFLTIGTPDEVNEQVAQYKALPVVLENARILKNCVDAKMTEEDKENALSLLDKINTSPLC (SEQ ID No 4)  
EICPAVKRDVVLFLTIGT (SEQ ID No 6)  
LFLTGTPDEVNEQVAQY (SEQ ID No 1)  
EQVAQYKALPVVLENA (SEQ ID No 2)  
KALPVVLENAIRLKNCY (SEQ ID No 3)  
RILKNCVDAKMTEEDKE (SEQ ID No 7)  
KMTEEDKENALSLLDK (SEQ ID No 8)  
KENALSVLDKIYTSP (SEQ ID No 9)

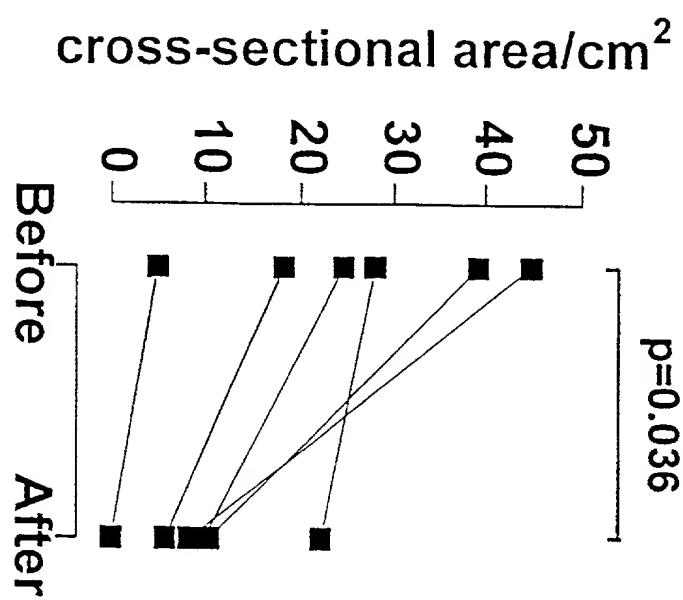
**Fel d I Chain 2**

VKVAVERCPIFYDVFFAVANGNELLKLSLTKVNATEPERTAMKKIQDCYYENGLISRVLDGLVMTISSLSSKDCMGEAVQNTVEDLKLNLTGR (SEQ ID No 5)  
VKVAVERCPIFYDVFFAVANGNELLKLSLTKVNATEPERTAMKKIQDCYYENGLISRVLDGLVMTISSLSSKDCMGEAVQNTVEDLKLNLTGR (SEQ ID No 5)  
CPIFYDVFFAVANGNEL (SEQ ID No 10)  
GNEELLKLSLTKVNAT (SEQ ID No 12)  
LTKVNATEFERTAMKK (SEQ ID No 13)  
TAMKKIQDCYYENGLI (SEQ ID No 14)  
YYENGLISRVLDGLY (SEQ ID No 15)  
SRYUDGLYMTISSLK (SEQ ID No 16)  
ISSSKDCMGEAVQNTV (SEQ ID No 17)  
AVQNTVEDLKLNLTGR (SEQ ID No 18)

Figure 10

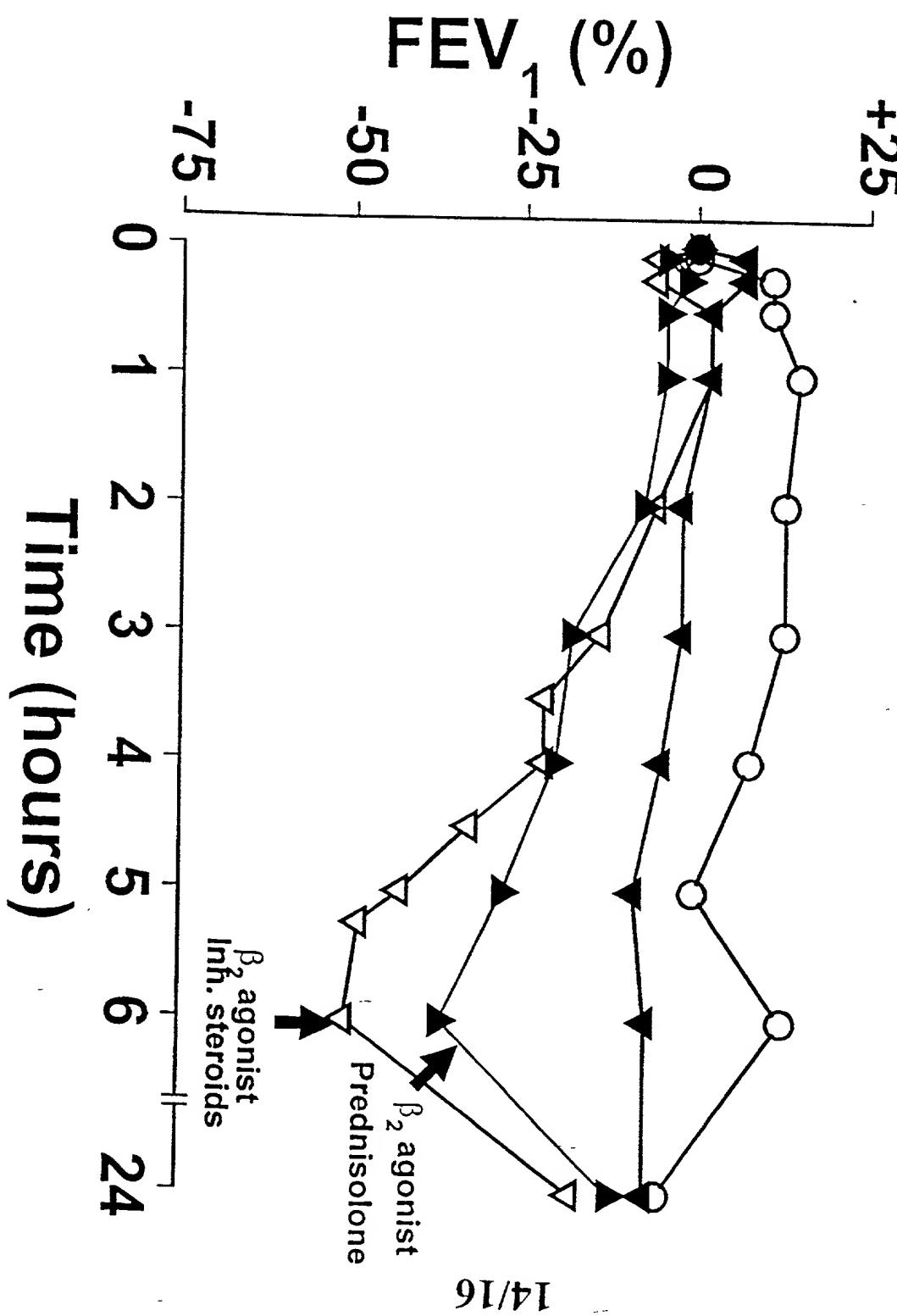


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**FIGURE 11**

Changes in the cutaneous late phase response  
to intradermal whole cat dander following  
intradermal administration of MOP

Figure 12a



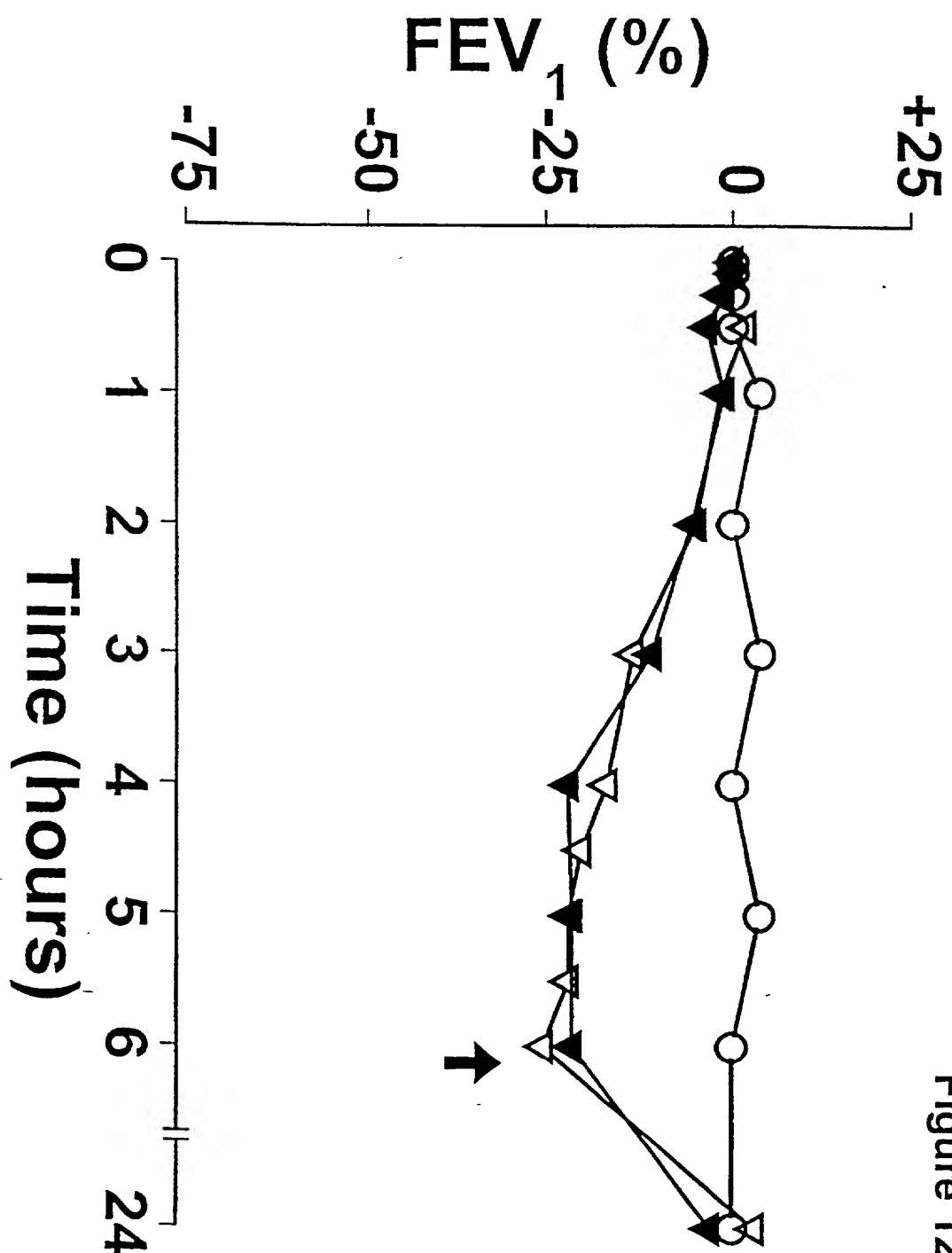
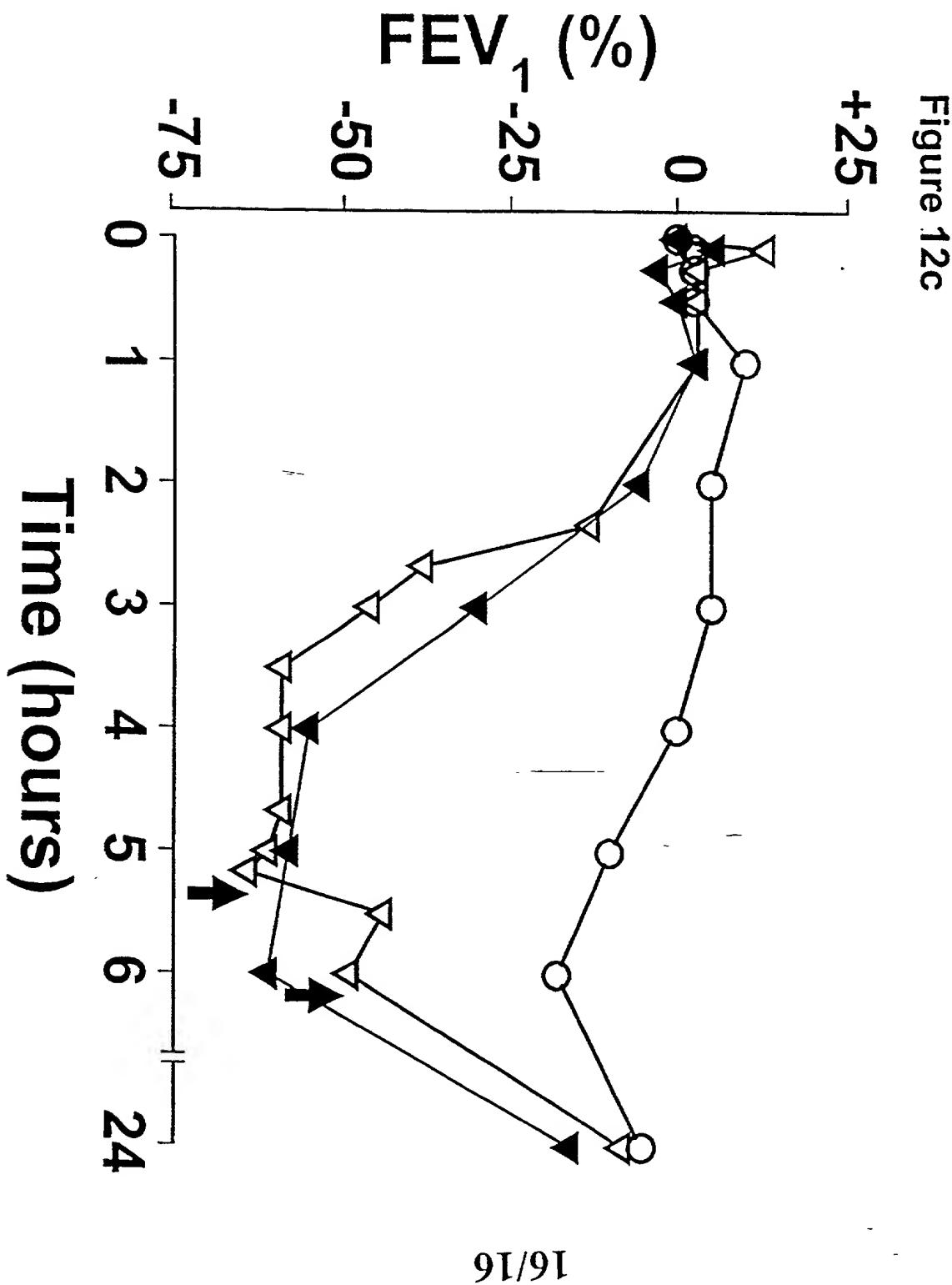


Figure 12b

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**DECLARATION AND POWER OF ATTORNEY  
FOR A PATENT APPLICATION**

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**METHODS AND COMPOSITIONS FOR DESENSITISATION**

the specification of which:

(check one)  is attached hereto.

was filed on \_\_\_\_\_ as  
Application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_  
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)		Priority Claimed	
<u>9800445.0</u> (Number)	<u>GB</u> (Country)	<u>9/1/98</u> (Day/Month/Year filed)	<input checked="" type="checkbox"/> [ ] Yes No
<u>9820474.6</u> (Number)	<u>GB</u> (Country)	<u>21/9/ 98</u> (Day/Month/Year filed)	<input checked="" type="checkbox"/> [ ] Yes No
<u>                  </u> (Number)	<u>                  </u> (Country)	<u>                  </u> (Day/Month/Year filed)	[ ] [ ] Yes No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Patent application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

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<u>PCT/GB99/00080</u> (Application Serial No.)	<u>11 January 1999</u> (Filing Date)	<u>PENDING</u> (Status: patented/pending/abandoned)
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<u>                  </u> (Application Serial No.)	<u>                  </u> (Filing Date)	<u>                  </u> (Status: patented/pending/abandoned)
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<u>                  </u> (Application Serial No.)	<u>                  </u> (Filing Date)	<u>                  </u> (Status: patented/pending/abandoned)
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As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Third Inventor's signature \_\_\_\_\_ Date \_\_\_\_\_

Residence \_\_\_\_\_

Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_